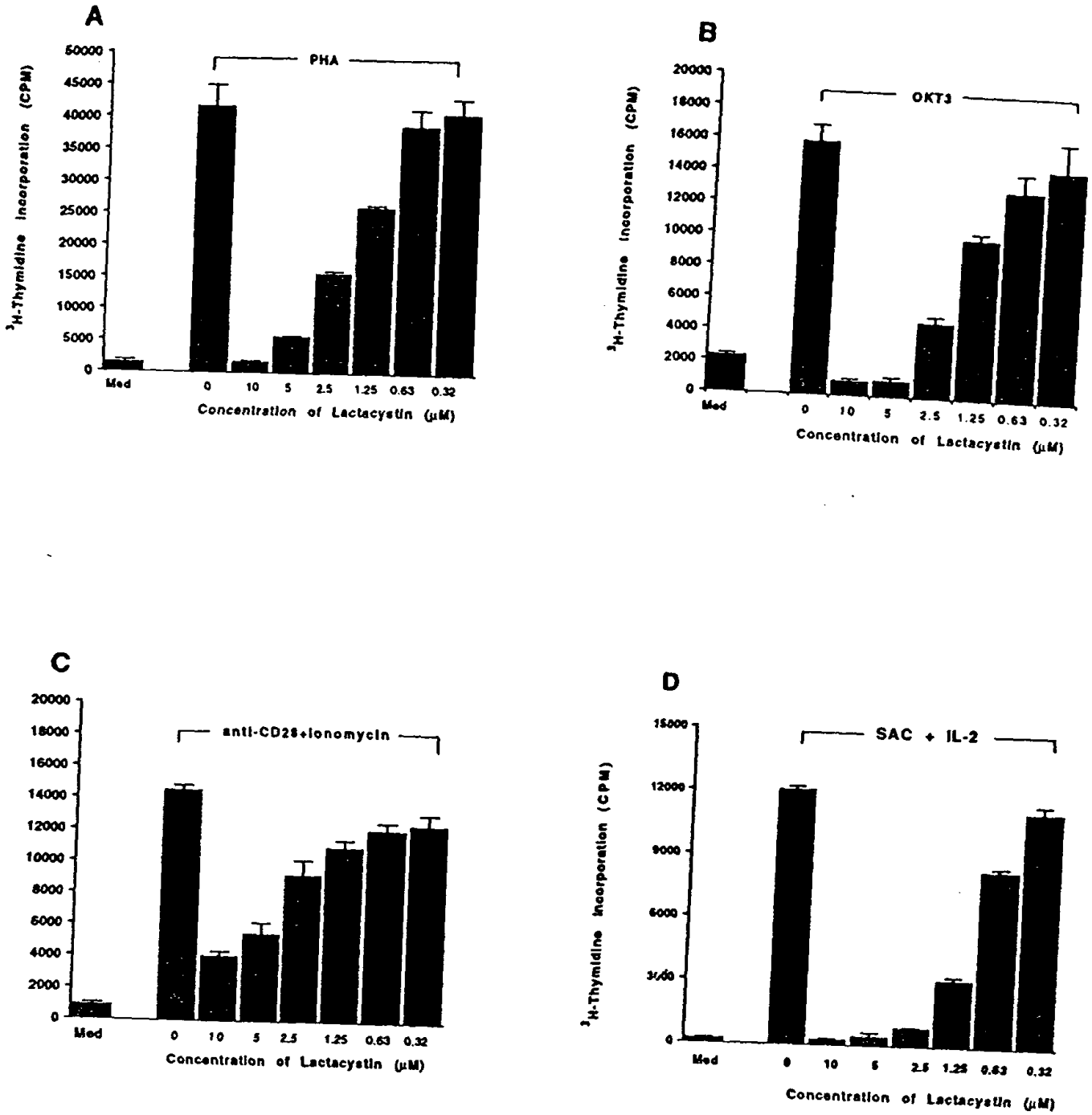

A
PHA
^{3}H-Thymidine Incorporation (CPM)
50000
45000
40000
35000
30000
25000
20000
15000
10000
5000
0
Med
0
10
5
2.5
1.25
0.63
0.32
Concentration of Lactacystin (μM)
B
OKT3
^{3}H-Thymidine Incorporation (CPM)
20000
18000
16000
14000
12000
10000
8000
6000
4000
2000
0
Med
0
10
5
2.5
1.25
0.63
0.32
Concentration of Lactacystin (μM)
C
anti-CD28+Ionomycin
^{3}H-Thymidine Incorporation (CPM)
20000
18000
16000
14000
12000
10000
8000
6000
4000
2000
0
Med
0
10
5
2.5
1.25
0.63
0.32
Concentration of Lactacystin (μM)
D
SAC + IL-2
^{3}H-Thymidine Incorporation (CPM)
15000
12000
9000
6000
3000
0
Med
0
10
5
2.5
1.25
0.63
0.32
Concentration of Lactacystin (μM)

FIGURE 1

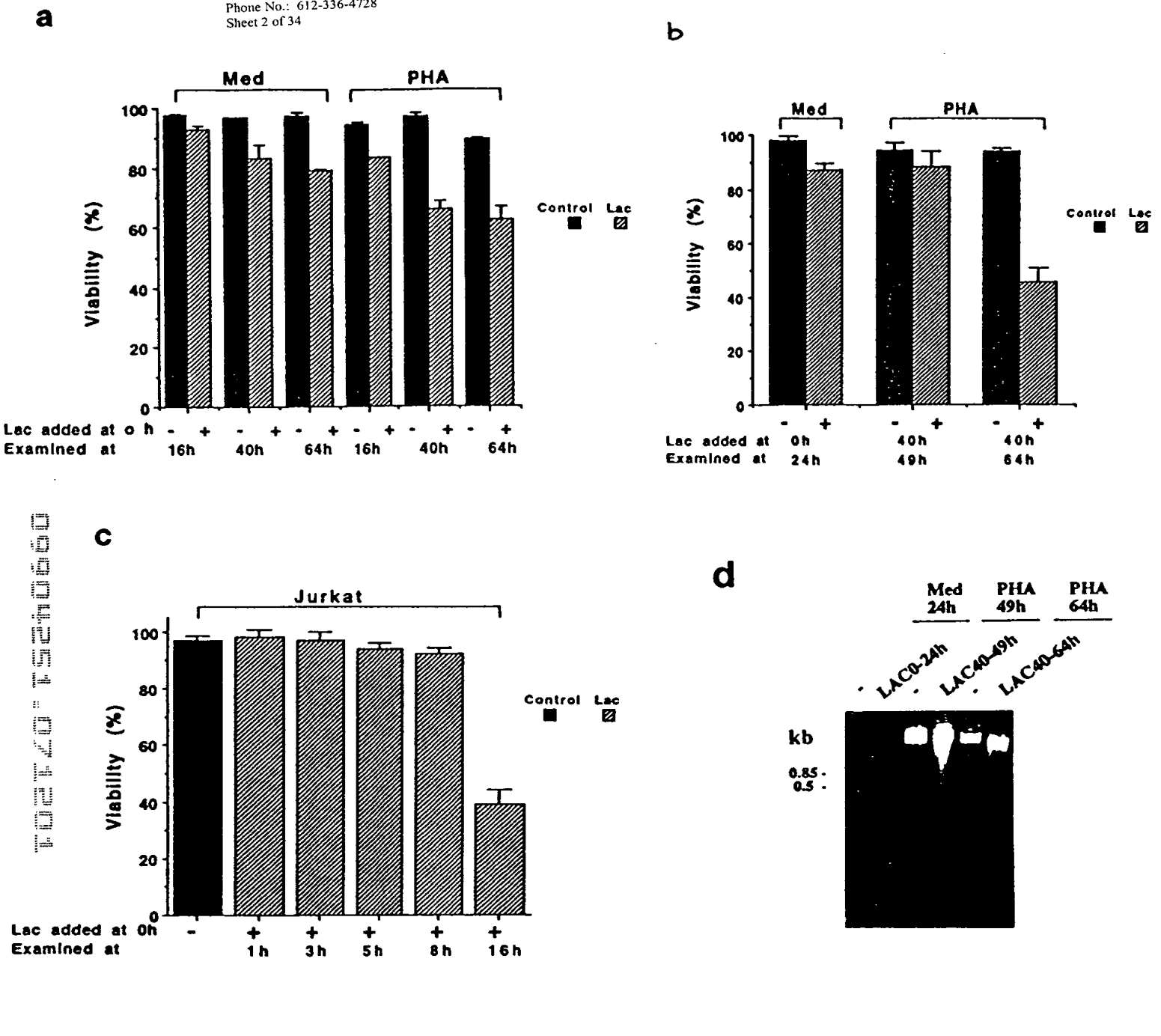

a
Med
PHA
Viability (%)
Control
Lac
Lac added at o h
Examined at
16h
40h
64h
b
Med
PHA
Viability (%)
Control
Lac
Lac added at
Examined at
0h
24h
40h
49h
40h
64h
c
Jurkat
Viability (%)
Control
Lac
Lac added at 0h
Examined at
1h
3h
5h
8h
16h
d
Med 24h
PHA 49h
PHA 64h
LAC0-24h
LAC40-49h
LAC40-64h
kb
0.85
0.5
e
Med
LAC6h
LAC16h
kb
0.85
0.5

FIGURE 2

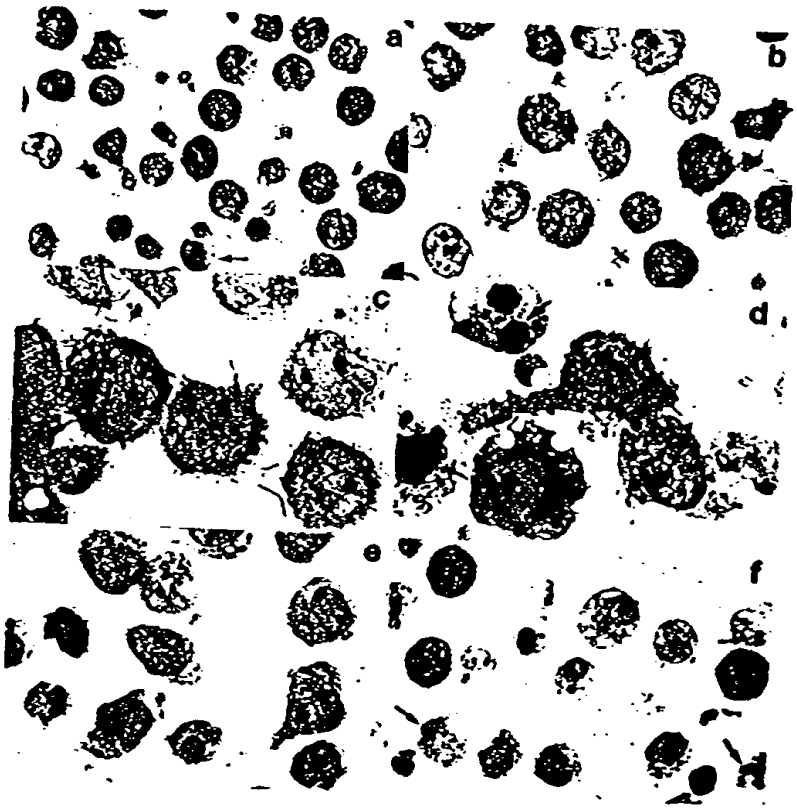
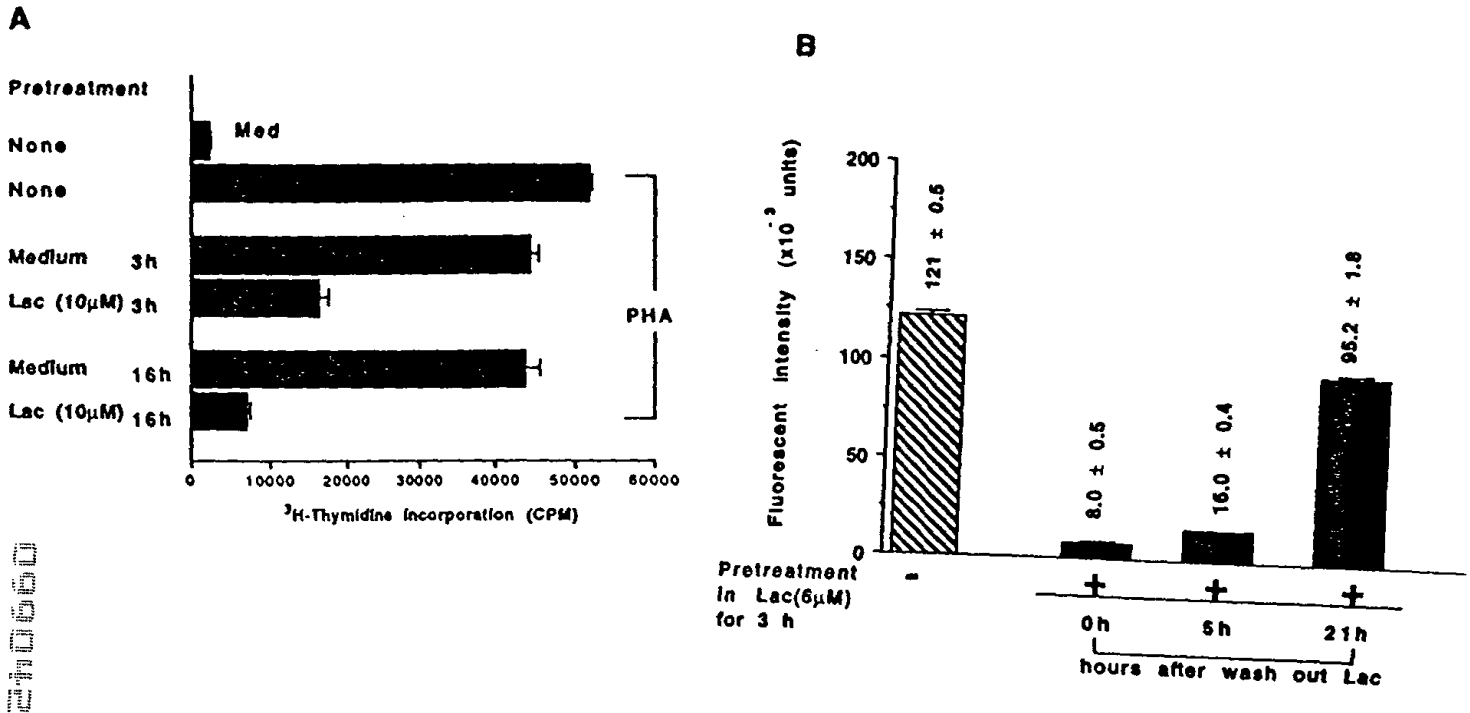


FIGURE 3


A
Pretreatment
None
None
Medium 3h
Lac (10μM) 3h
Medium 16h
Lac (10μM) 16h
Med
PHA
0
10000
20000
30000
40000
50000
60000
³H-Thymidine incorporation (CPM)
B
Fluorescent Intensity (x10⁻³ units)
200
150
100
50
0
121 ± 0.5
8.0 ± 0.5
16.0 ± 0.4
95.2 ± 1.8
Pretreatment in Lac(6μM) for 3 h
-
+
+
+
0h
5h
21h
hours after wash out Lac

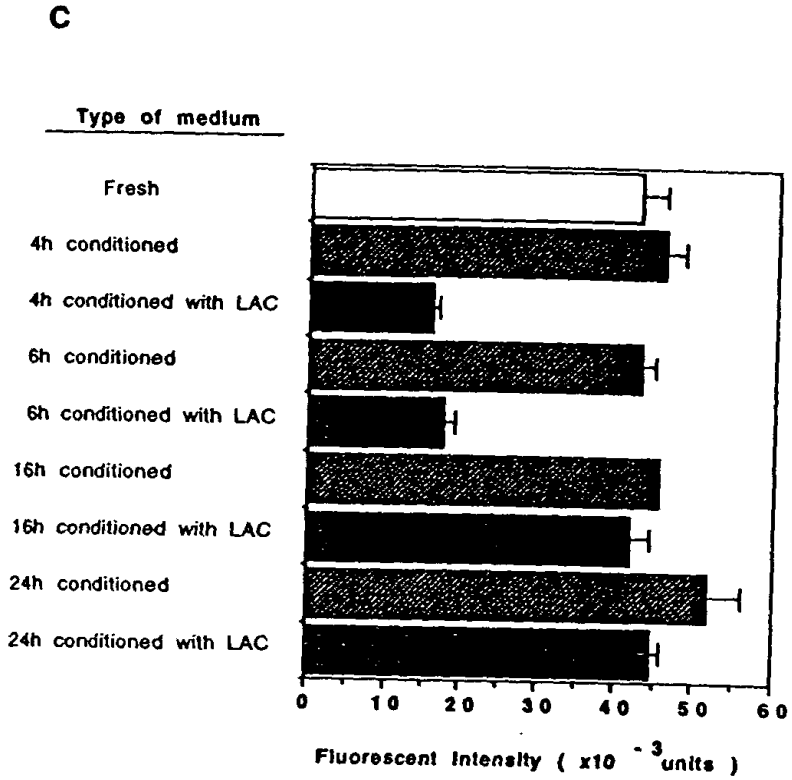
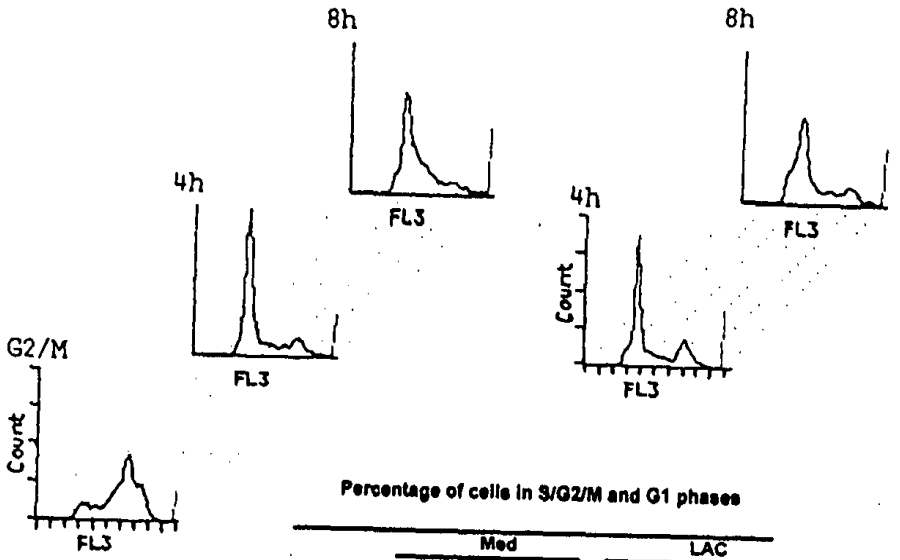

C
Type of medium
Fresh
4h conditioned
4h conditioned with LAC
6h conditioned
6h conditioned with LAC
16h conditioned
16h conditioned with LAC
24h conditioned
24h conditioned with LAC
0
10
20
30
40
50
60
Fluorescent Intensity (x10⁻³ units)

FIGURE 4

a

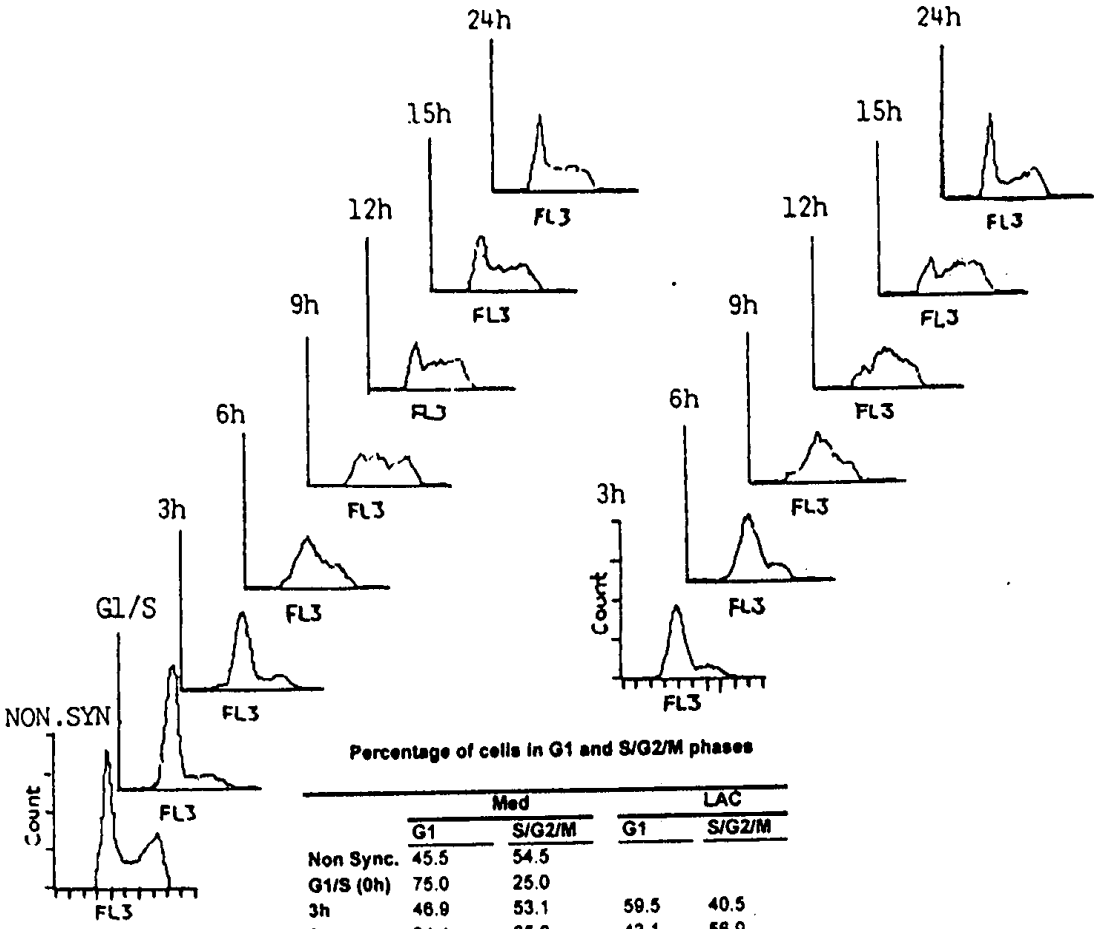


Percentage of cells in S/G2/M and G1 phases

	Med		LAC	
	S/G2/M	G1	S/G2/M	G1
G2/M (0h)	82.6	17.4		
4h	33.0	67.0	40.2	59.8
8h	41.9	58.1	36.5	63.5

FIGURE 6A

b



Percentage of cells in G1 and S/G2/M phases

	Med		LAC	
	G1	S/G2/M	G1	S/G2/M
Non Sync.	45.5	54.5		
G1/S (0h)	75.0	25.0		
3h	46.9	53.1	59.5	40.5
6h	34.4	65.6	43.1	56.9
9h	30.3	69.7	25.2	74.8
12h	42.3	57.7	27.8	72.2
15h	46.9	53.1	30.7	69.3
20h	56.0	44.0	55.7	44.3

FIGURE 6B

C

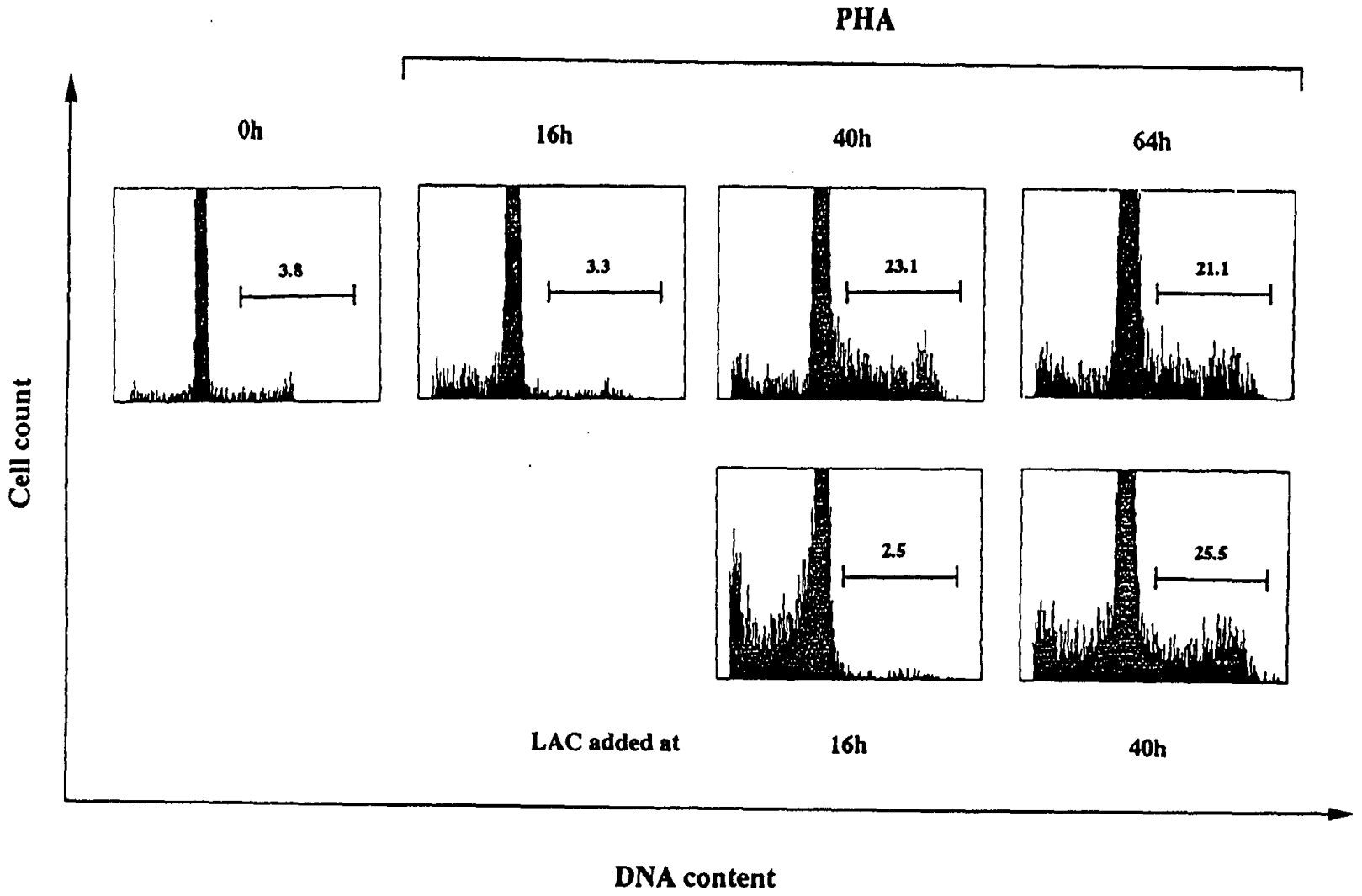


FIGURE 6C

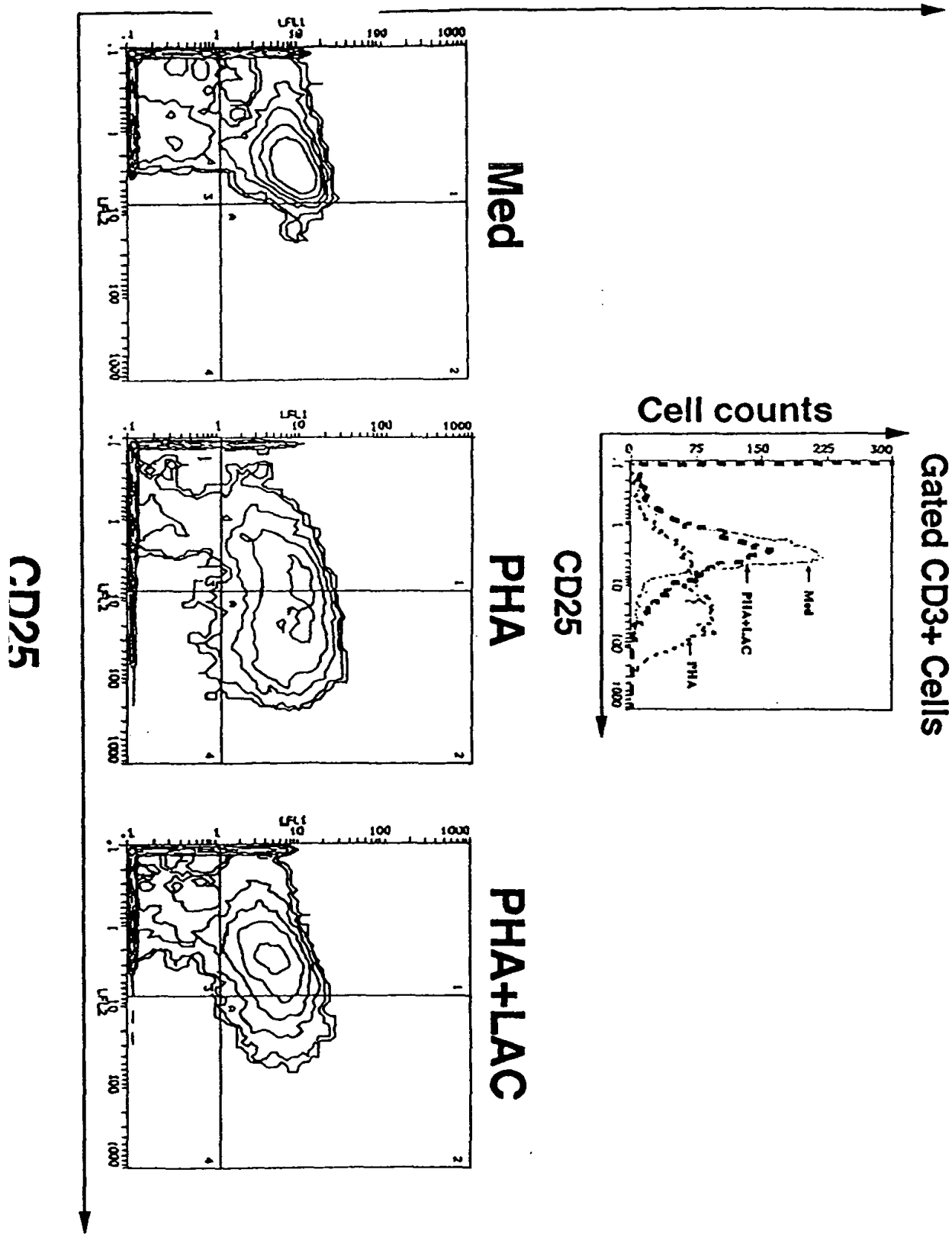

CD3
CD25
Med
PHA
PHA+LAC
Cell counts
Gated CD3+ Cells
CD25
Med
PHA+LAC
PHA

FIGURE 5

d

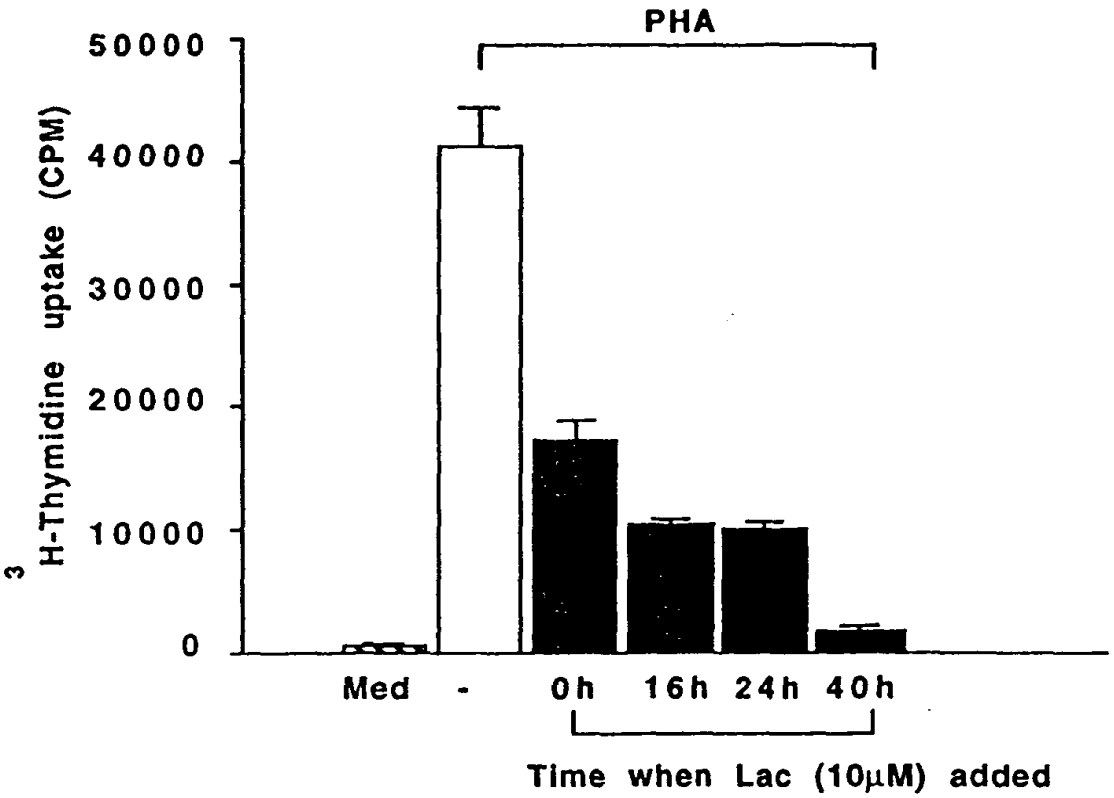

PHA
50000
40000
30000
20000
10000
0
^{3}H-Thymidine uptake (CPM)
Med
-
0h
16h
24h
40h
Time when Lac (10μM) added

FIGURE 6D

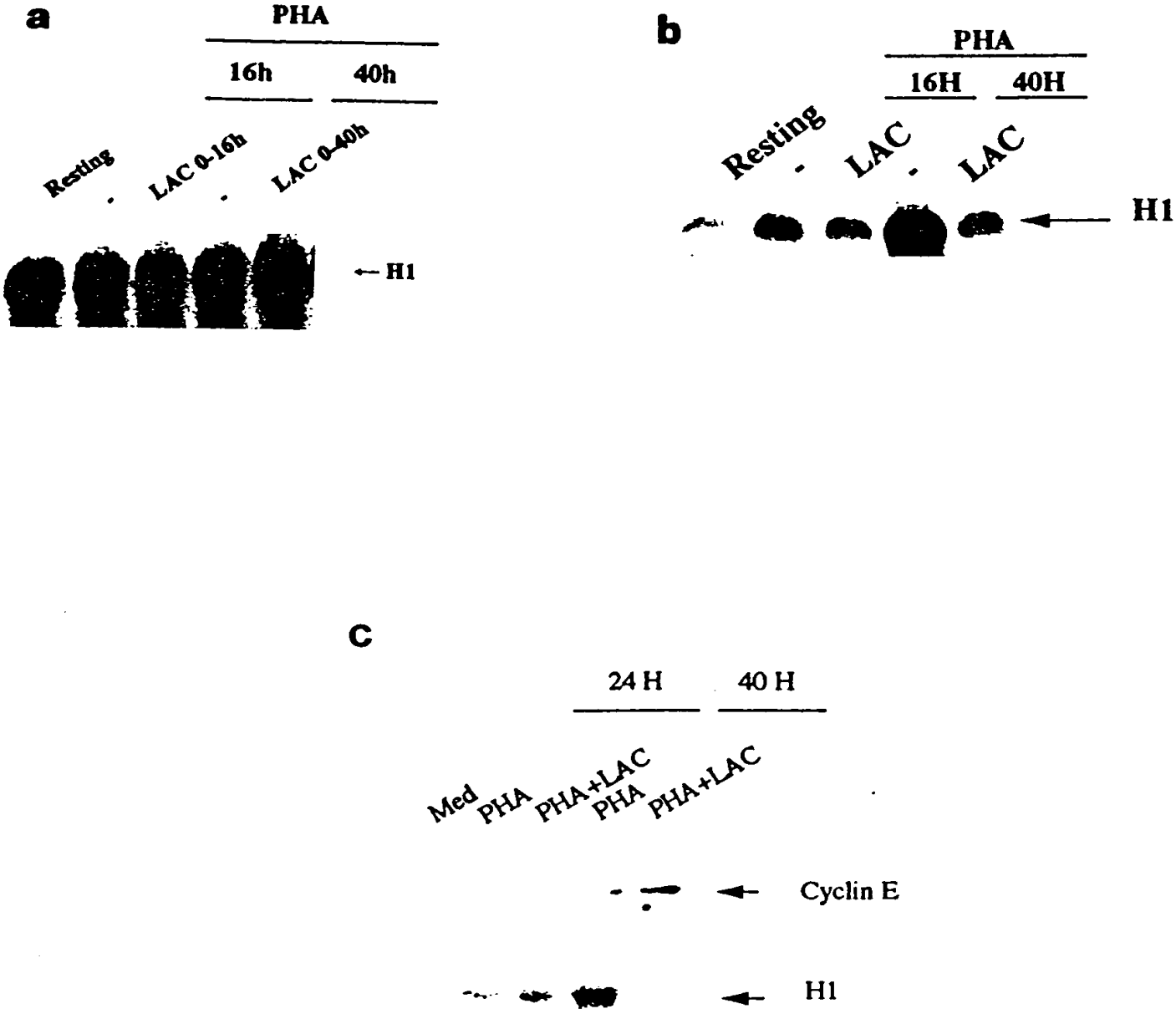
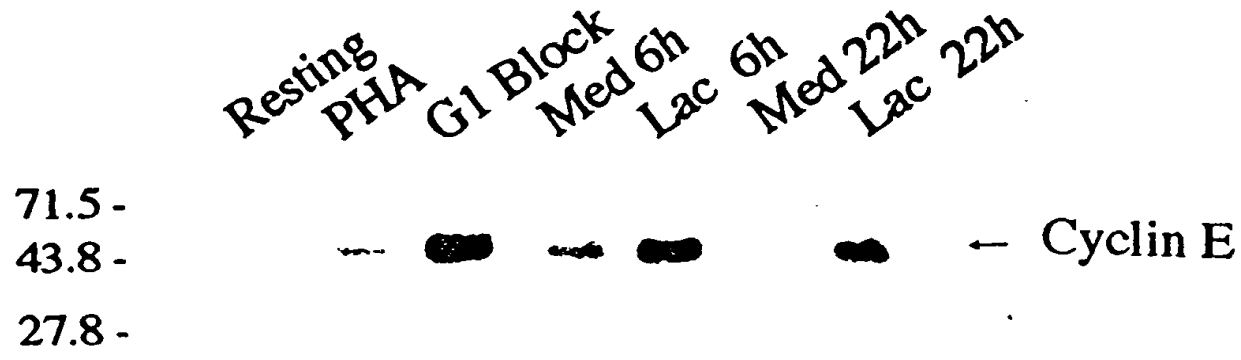

a
PHA
16h
40h
Resting
LAC 0-16h
LAC 0-40h
H1
b
PHA
16H
40H
Resting
LAC
LAC
H1
c
24 H
40 H
Med
PHA
PHA+LAC
PHA
PHA+LAC
Cyclin E
H1

FIGURE 7

a.

Post G1 Block



b.

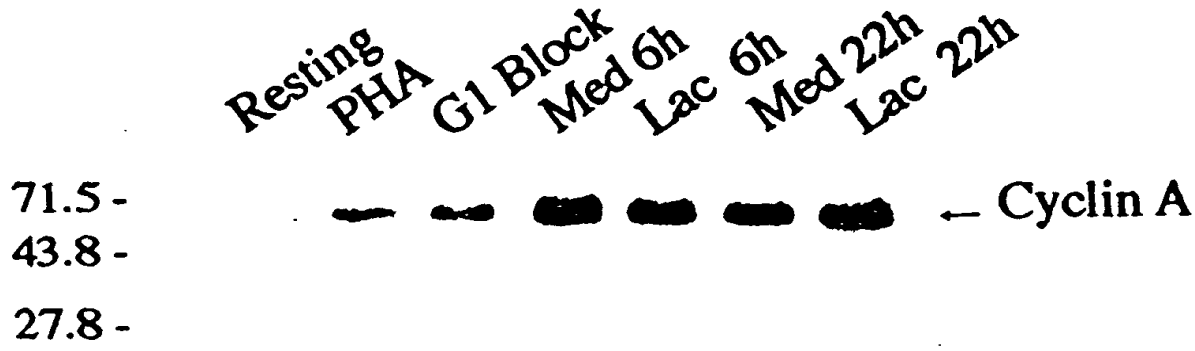
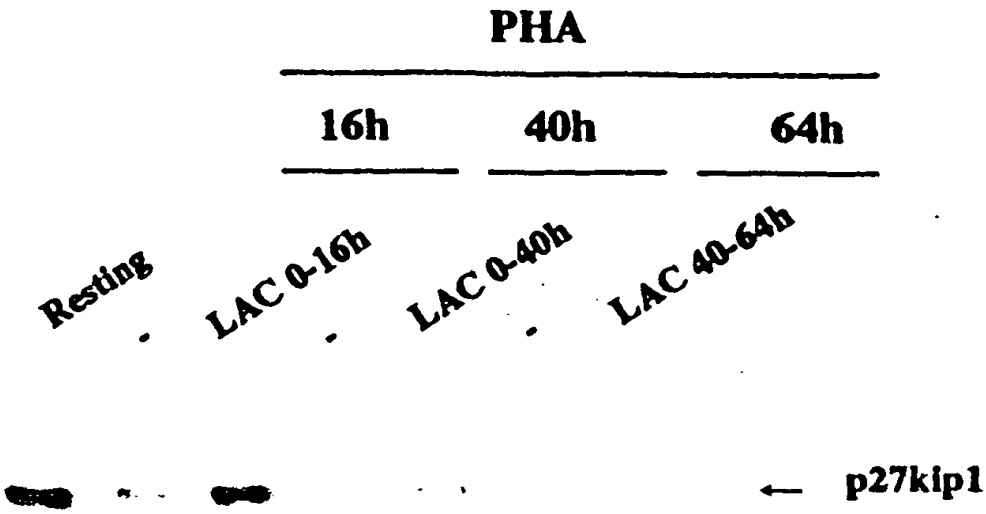


FIGURE 8

a.


PHA
16h
40h
64h
Resting
LAC 0-16h
LAC 0-40h
LAC 40-64h
p27kip1

b.

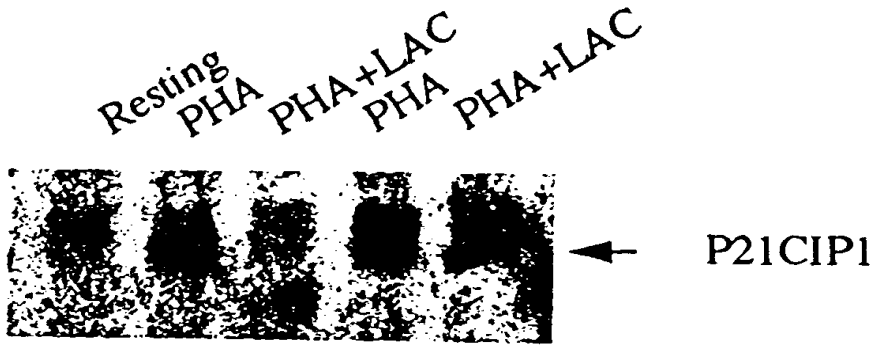

16 H
40 H
Resting
PHA
PHA+LAC
PHA
PHA+LAC
P21CIP1

FIGURE 9

Lactacystin inhibits aggregation of PHA-stimulated human PBMC

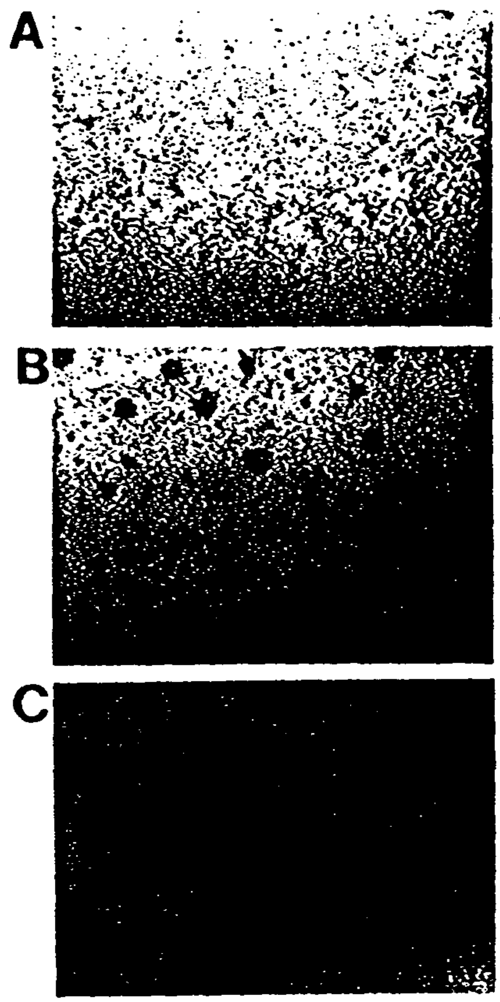


FIGURE 10

Lactacystin inhibits aggregation of mitogen-stimulated mouse lymph node cells

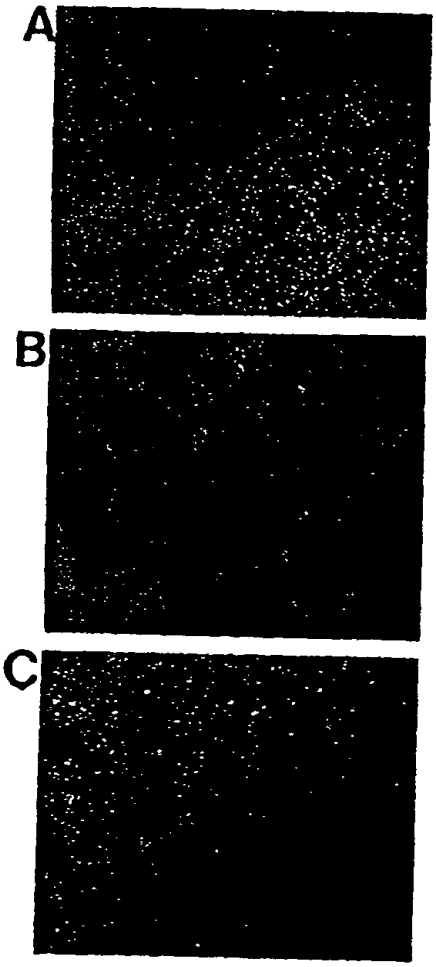


FIGURE 11

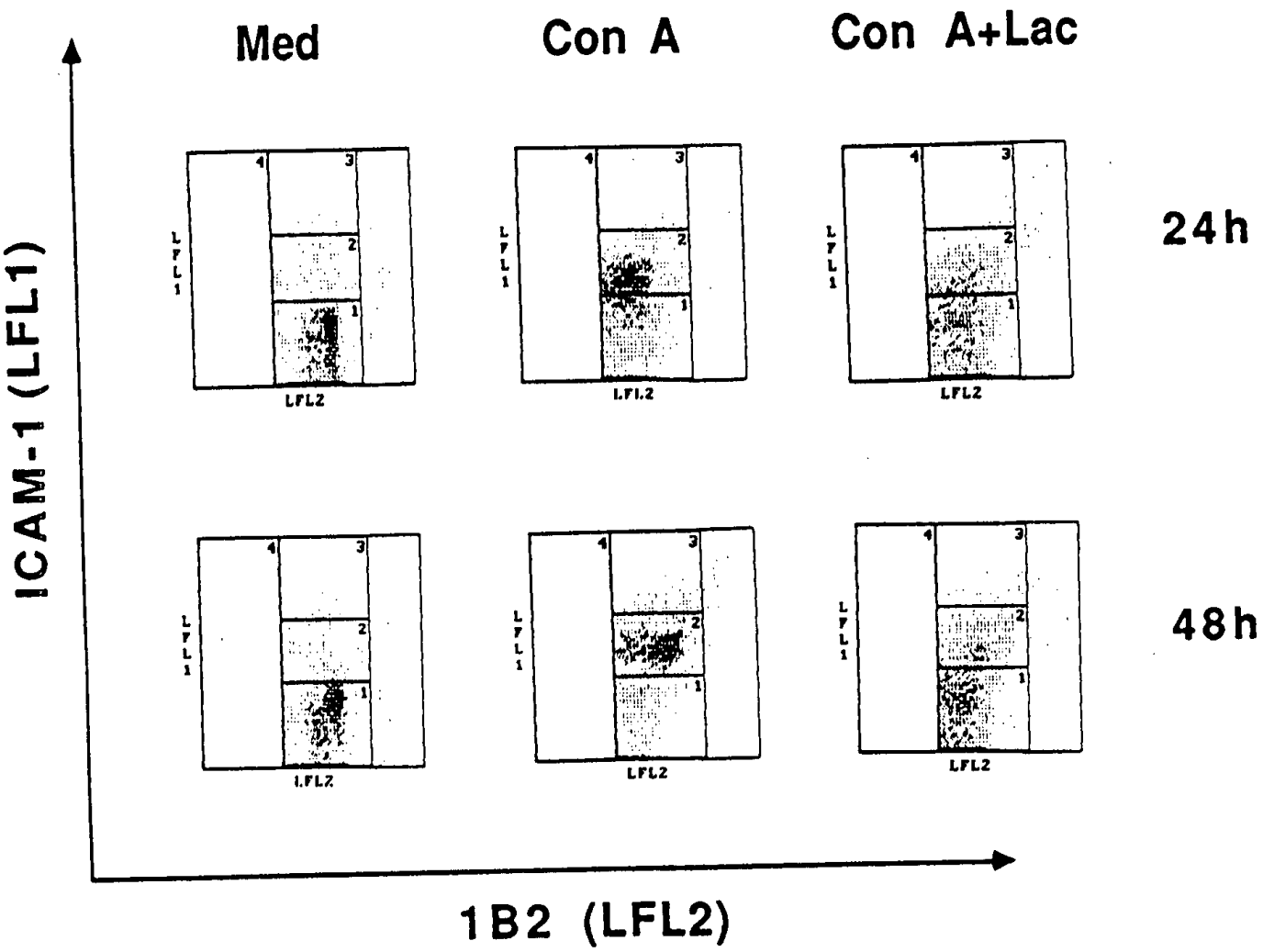


FIGURE 12

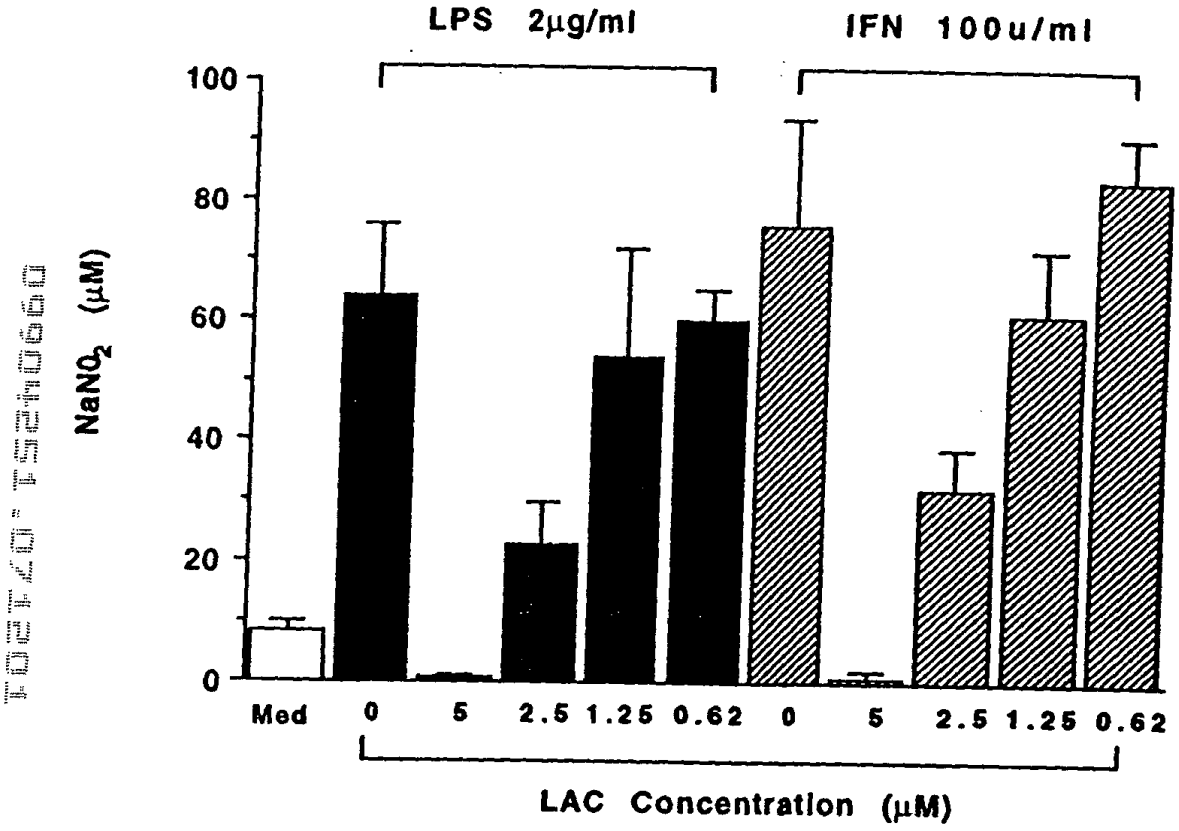

LPS 2μg/ml
IFN 100u/ml
100
80
60
40
20
0
NaNO2 (μM)
Med
0
5
2.5
1.25
0.62
0
5
2.5
1.25
0.62
LAC Concentration (μM)

FIGURE 13

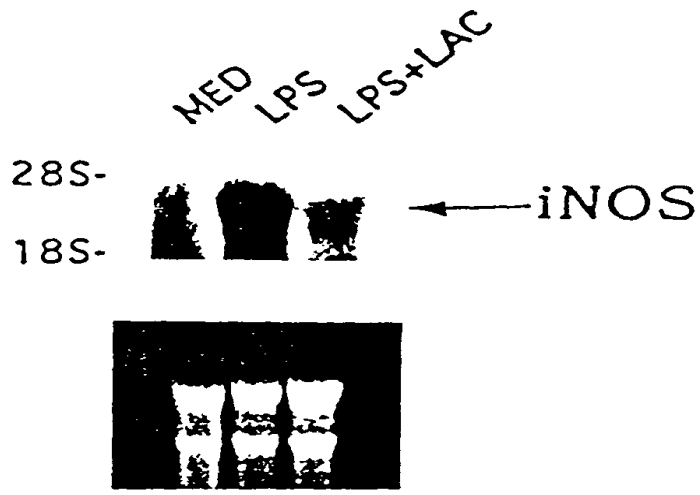


FIGURE 14

FIGURE 15

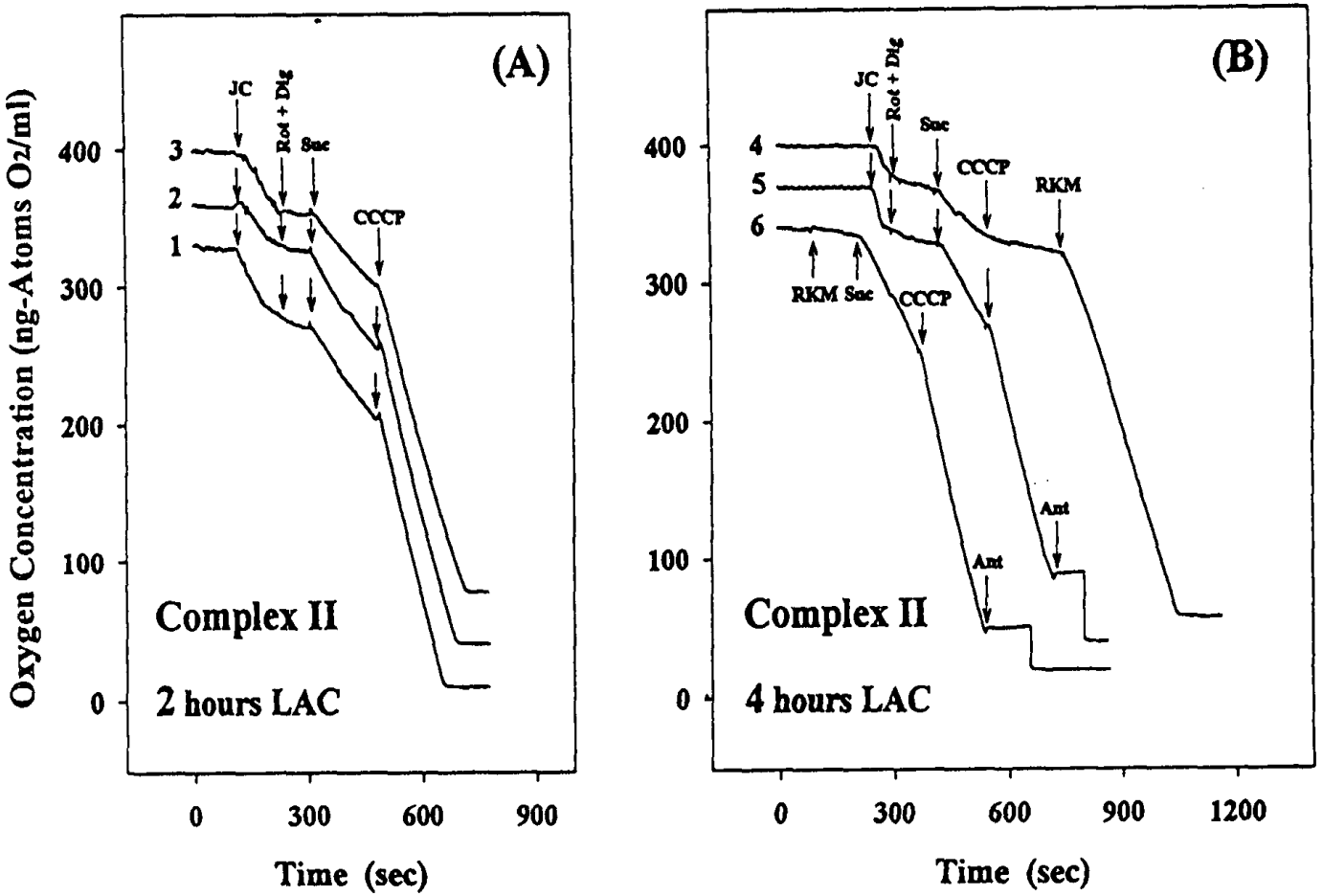


FIGURE 16

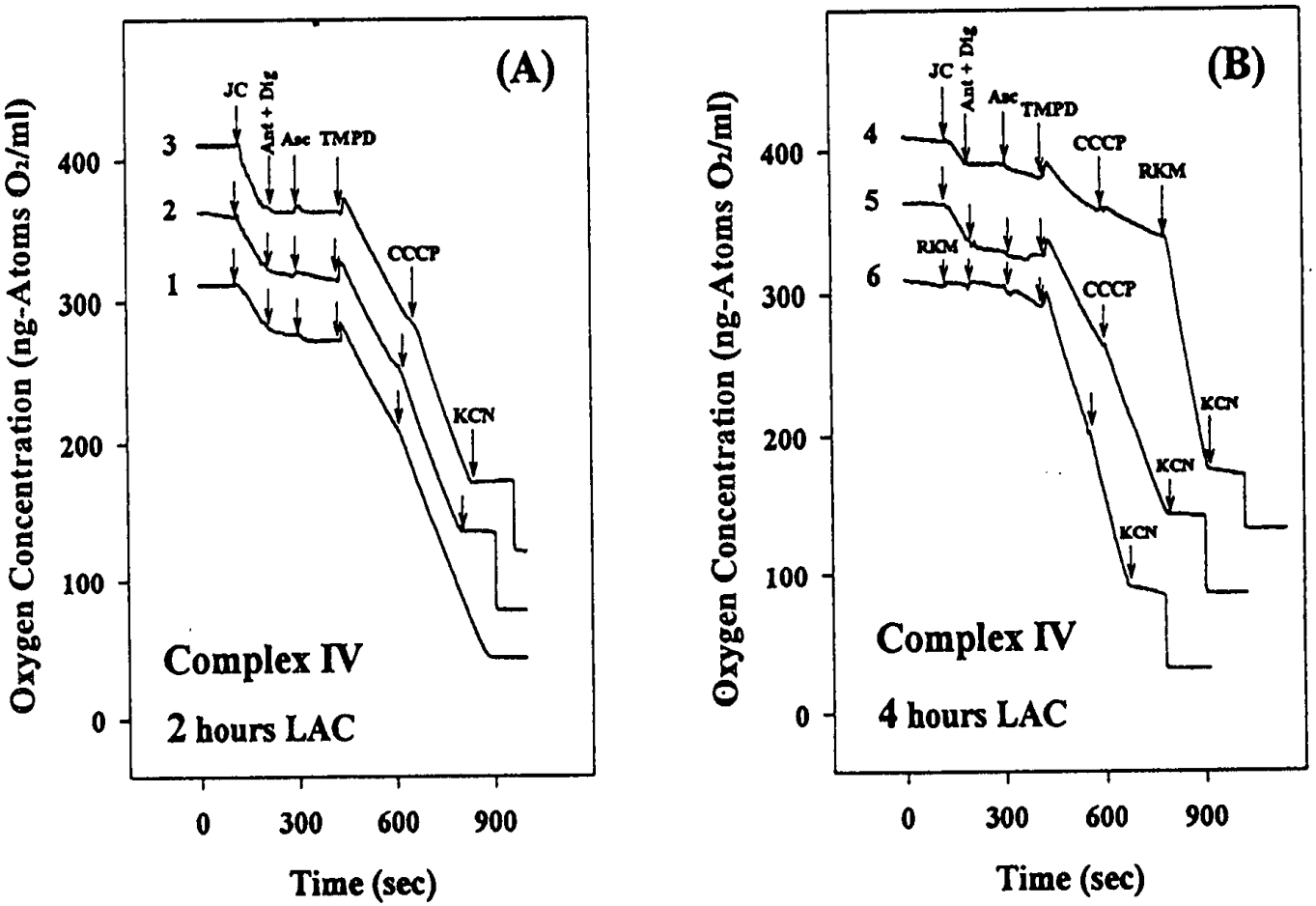
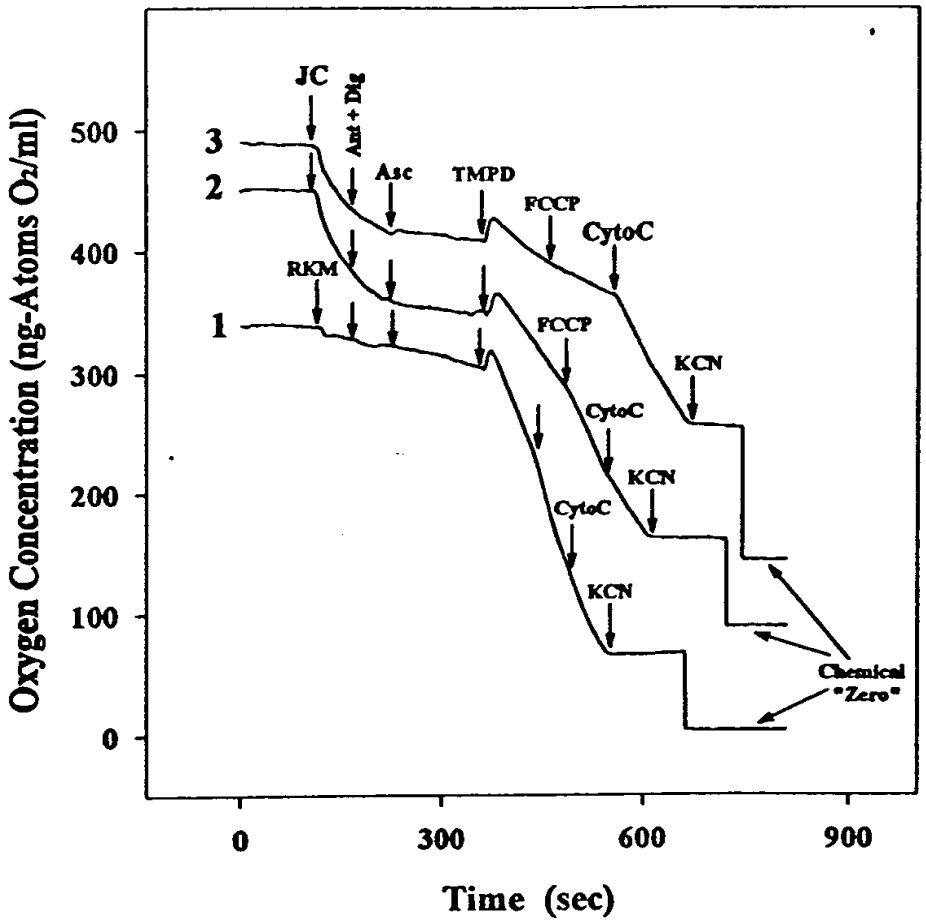


FIGURE 17


JC
Ant + Dig
Asc
TMPD
FCCP
CytoC
KCN
RKM
3
2
1
Chemical "Zero"
Oxygen Concentration (ng-Atoms O2/ml)
500
400
300
200
100
0
0
300
600
900
Time (sec)

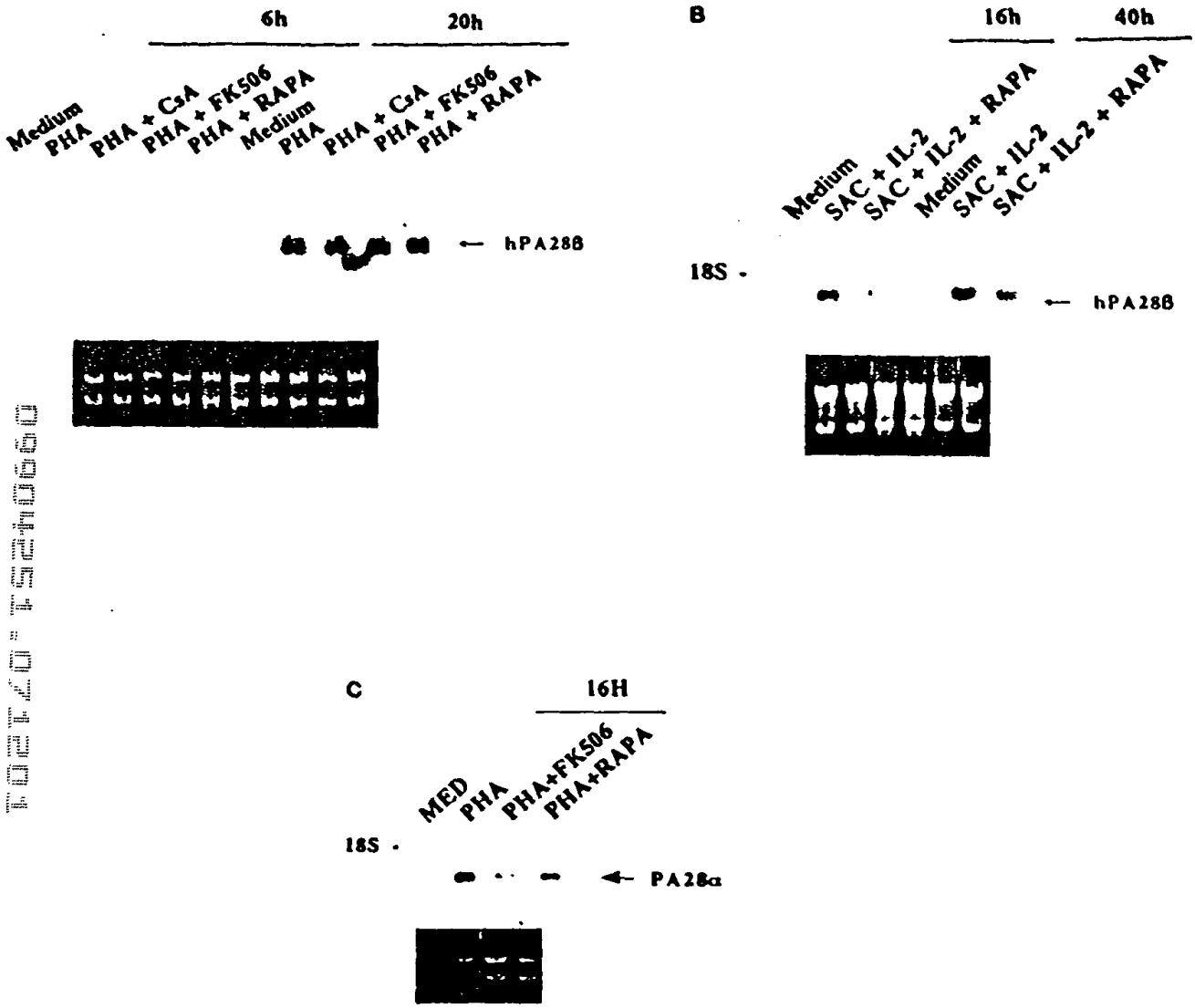

6h
20h
Medium
PHA
PHA + CsA
PHA + FK506
PHA + RAPA
Medium
PHA
PHA + CsA
PHA + FK506
PHA + RAPA
hPA28β
B
16h
40h
Medium
SAC + IL-2
SAC + IL-2 + RAPA
Medium
SAC + IL-2
SAC + IL-2 + RAPA
18S
hPA28β
C
16H
MED
PHA
PHA+FK506
PHA+RAPA
18S
PA28α

FIGURE 18

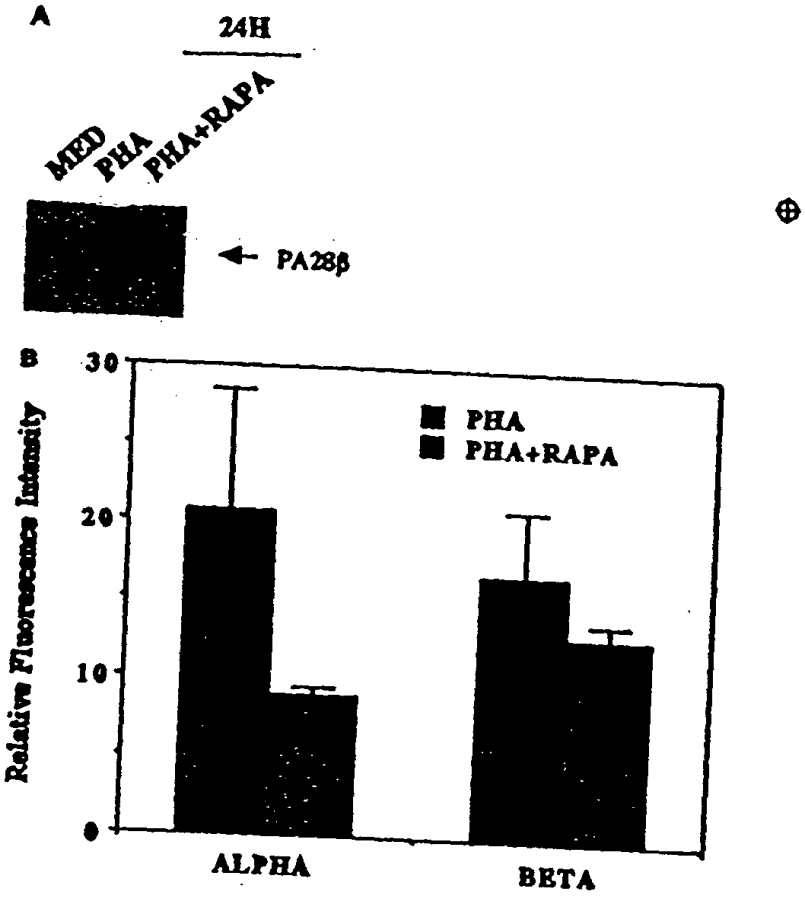

A
24H
MED
PHA
PHA+RAPA
PA28β
B
Relative Fluorescence Intensity
30
20
10
0
PHA
PHA+RAPA
ALPHA
BETA

FIGURE 19

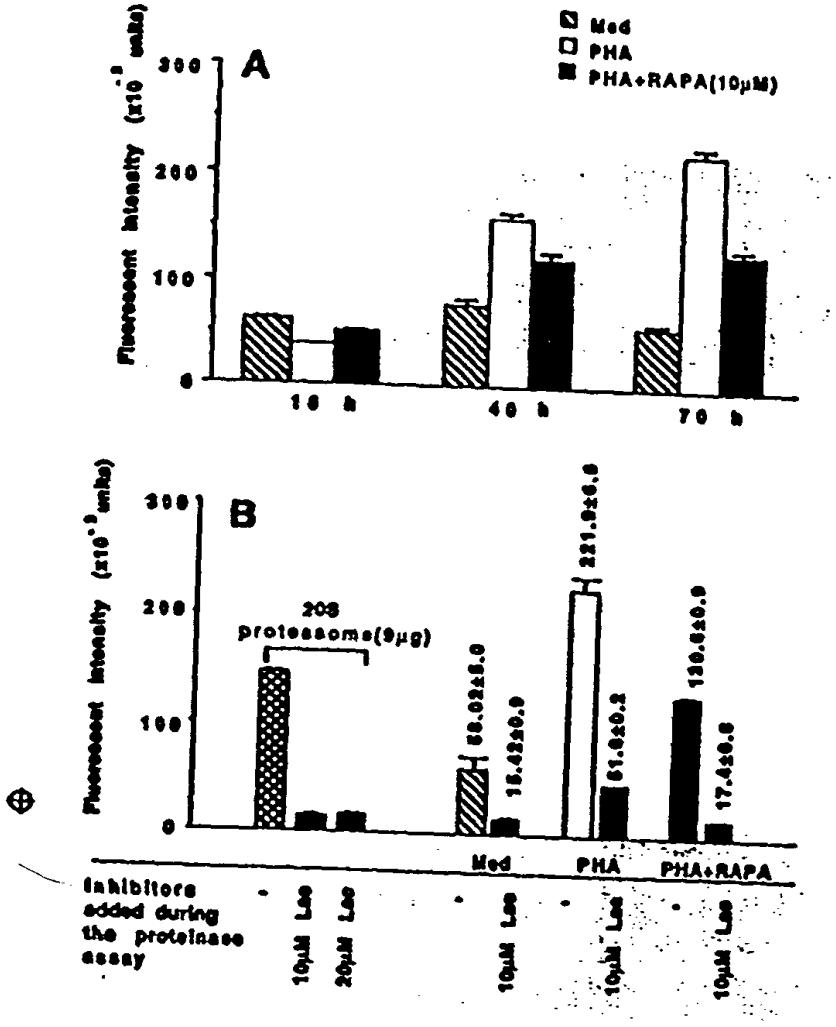

A
Med
PHA
PHA+RAPA(10μM)
Fluorescent intensity (x10⁻³ units)
300
200
100
0
16 h
40 h
70 h
B
20S proteasome(9μg)
58.0±5.0
15.4±0.9
221.9±6.8
61.0±0.2
130.6±0.9
17.4±0.8
Med
PHA
PHA+RAPA
Inhibitors added during the proteinase assay
10μM Leu
20μM Leu

FIGURE 20

Eliminating Alloantigen-specific Response by a Proteasome Inhibitor

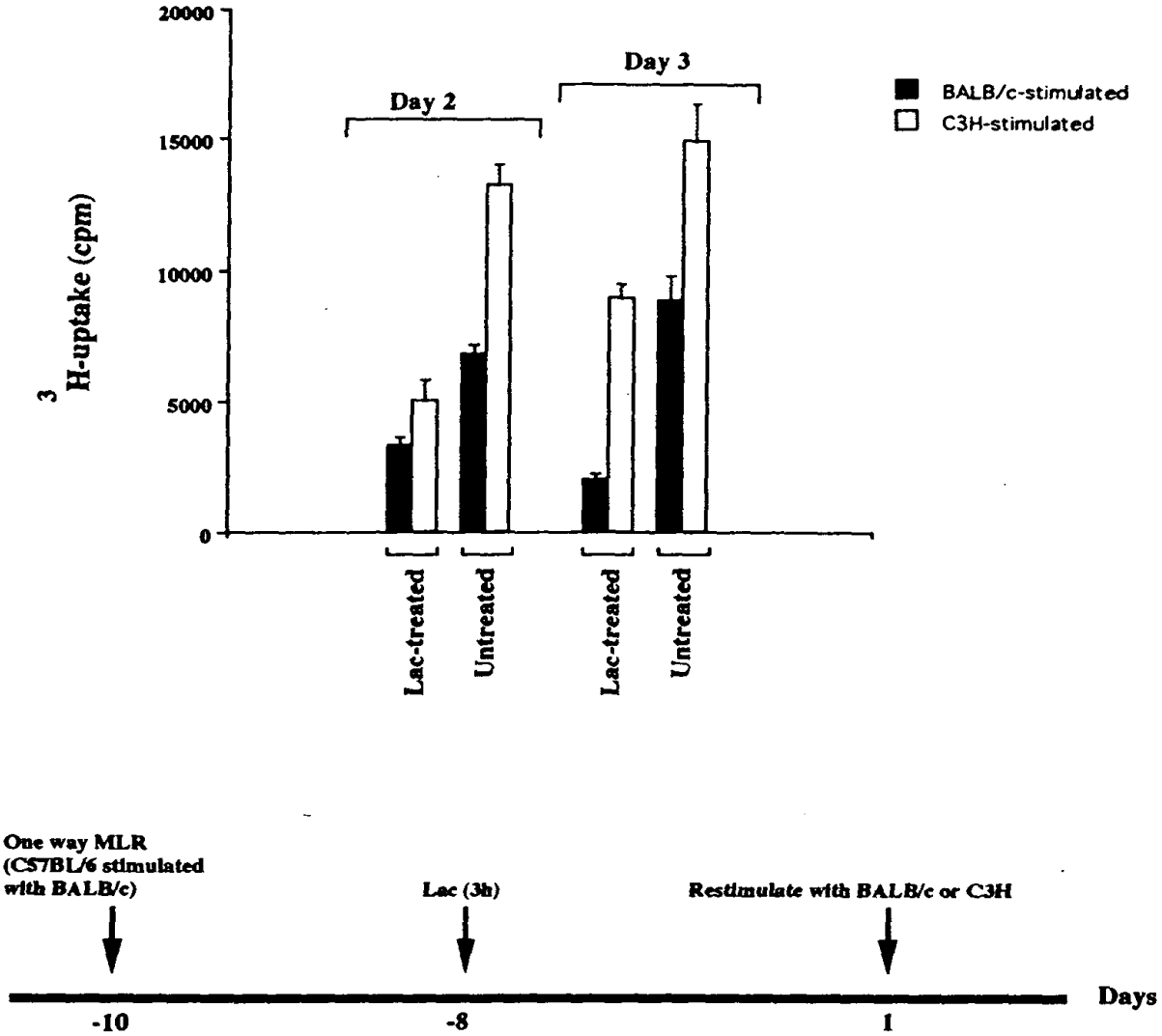


Fig. 21

Z-VAD.fmk blocks Lactacystin induced apoptosis in Jurkat cell

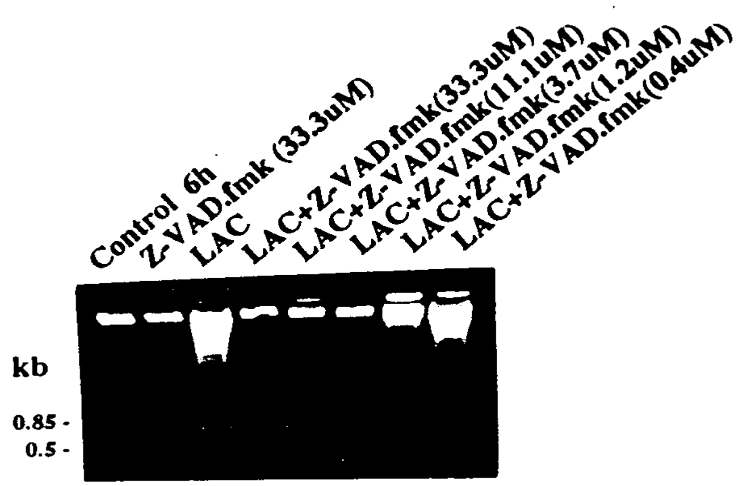


Fig. 22

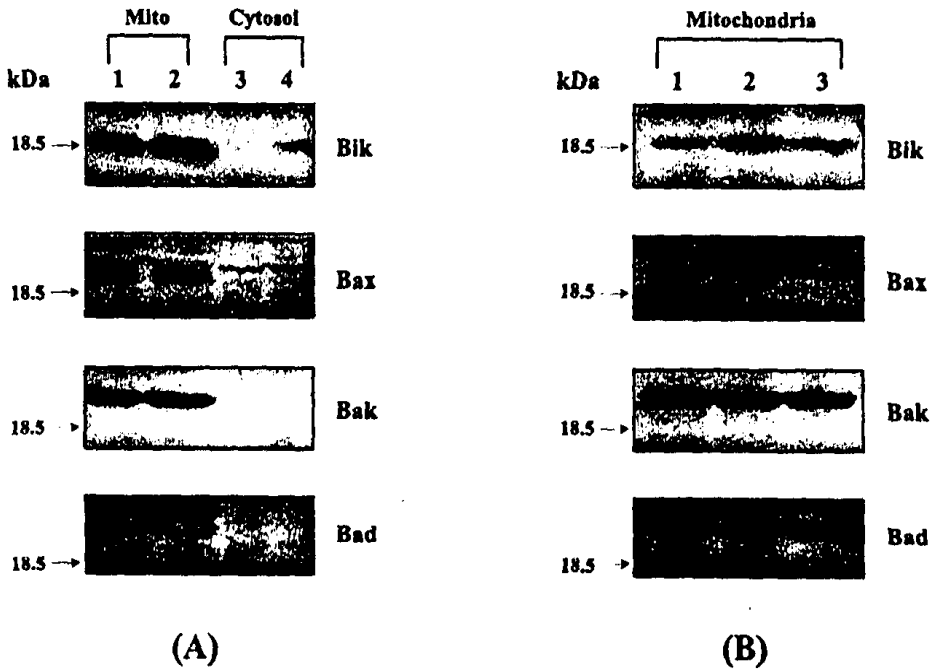


Fig. 23

DNA-fragmentation in Namalwa-Control (▒) and Namalwa-BclX$_L$ (■) cells during Lactacystin (LAC) induced apoptosis.

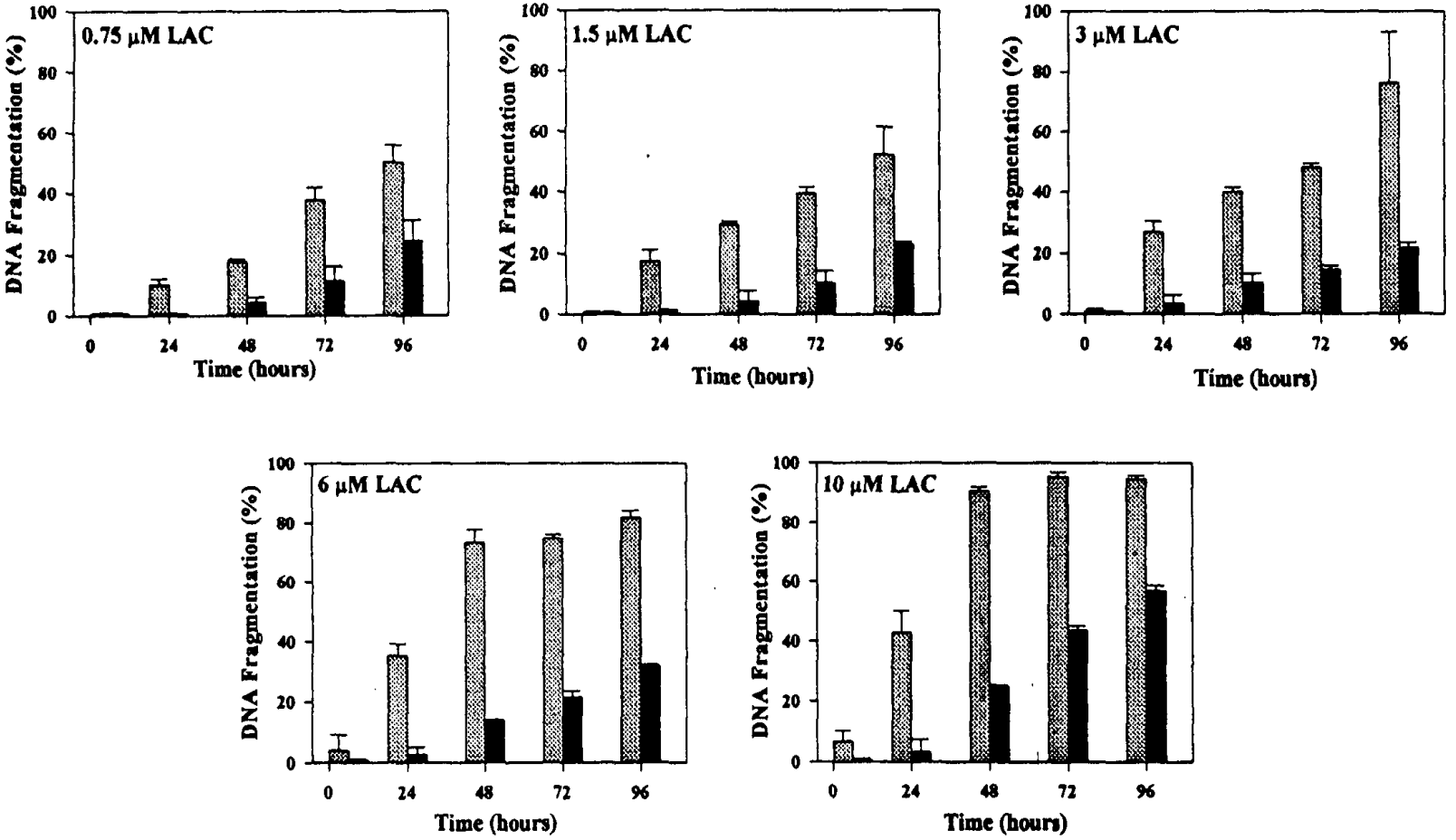


Fig. 24

Inventor: WU
Docket No.: 9555.94USI1
Title: USE OF PROTEASOME INHIBITORS FOR TREATING CANCER, INFLAMMATION, AUTOIMMUNE DISEASE, GRAFT REJECTION AND SEPTIC SHOCK
Attorney Name: Gregory A. Sebald
Phone No.: 612-336-4728
Sheet 27 of 34

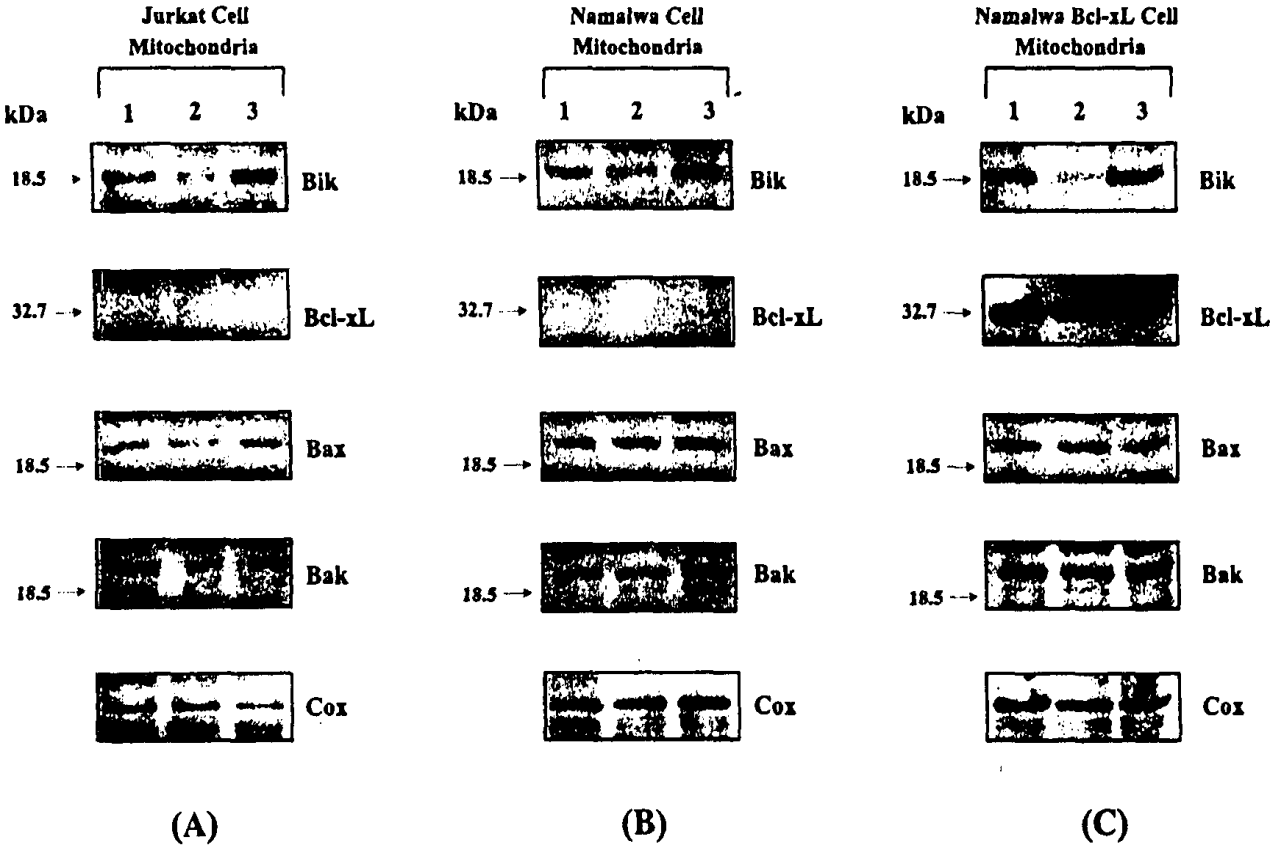
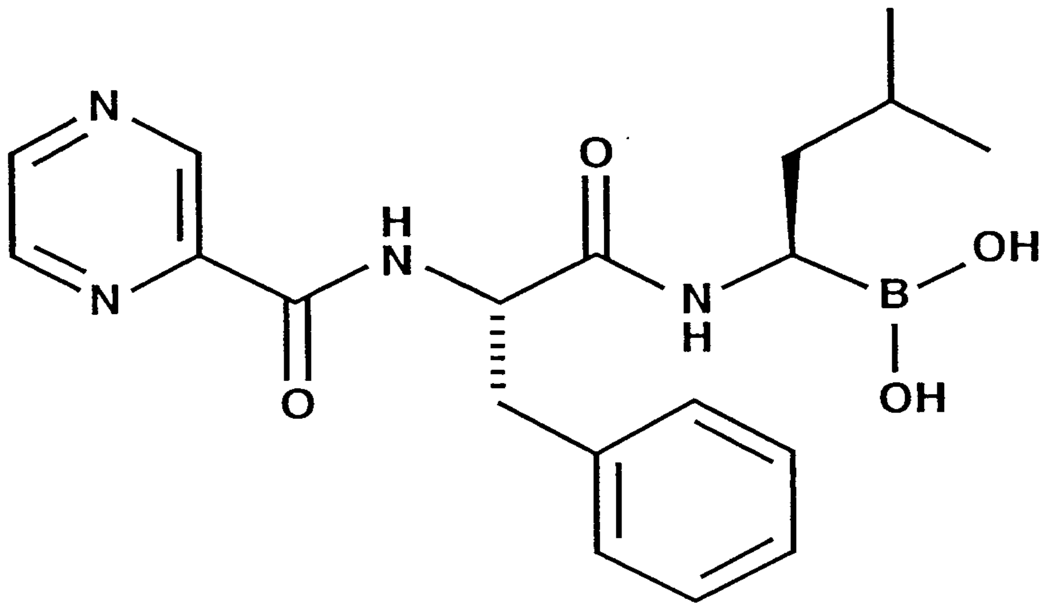


Fig. 25

DIPEPTIDE BORONIC ACID
(DPBA)

Pyz-Phe-boroLeu; Pyz, 2,5-pyrazinecarboxylic acid



LACTACYSTIN

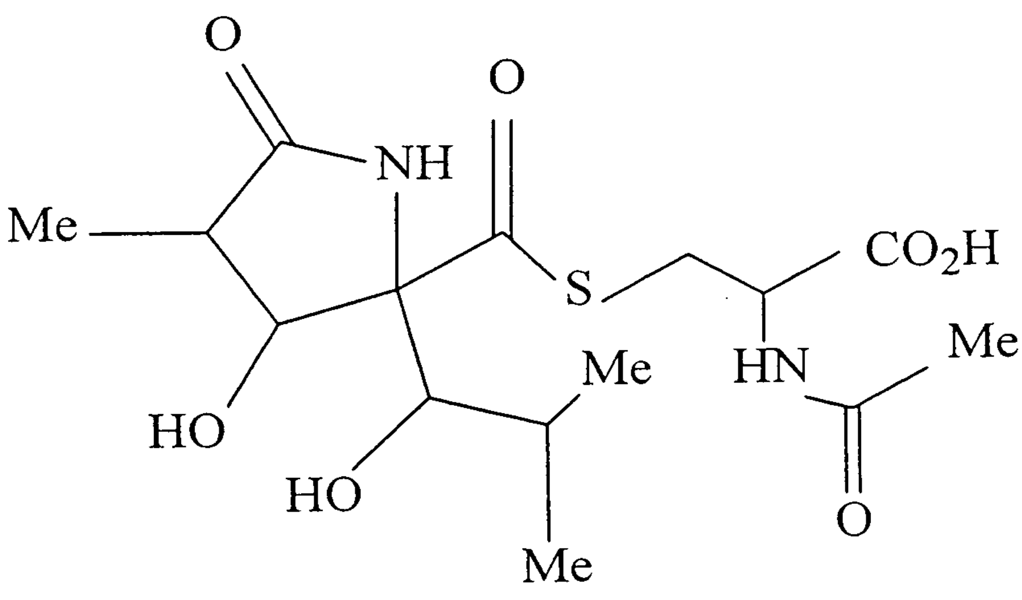


FIGURE 26

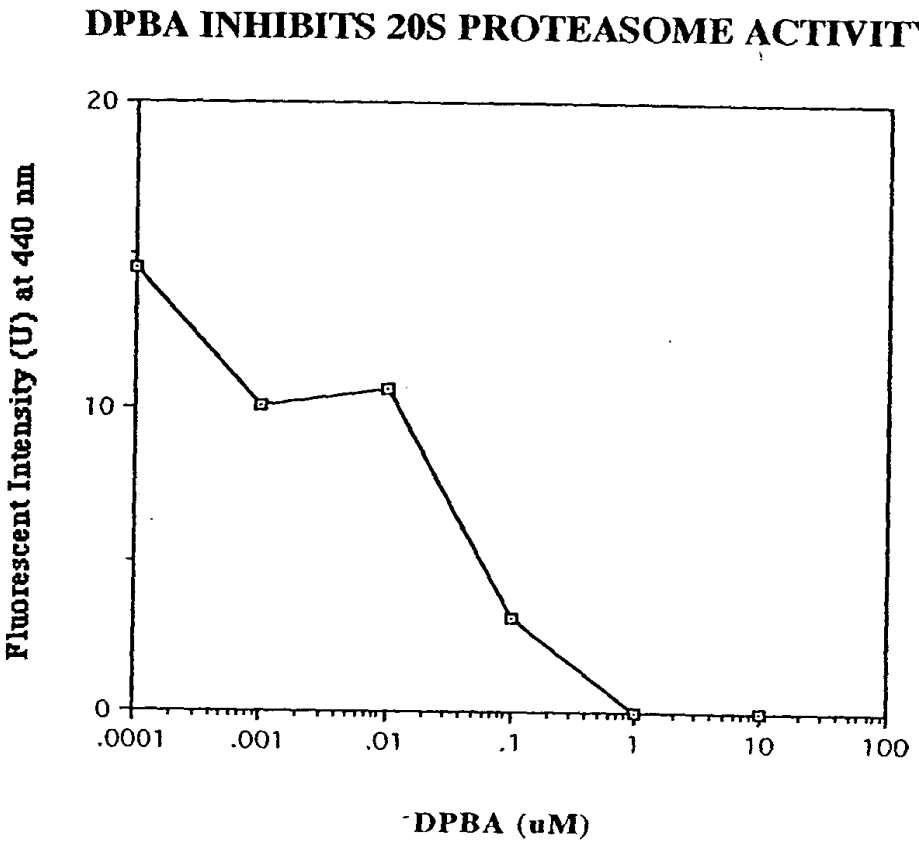

DPBA INHIBITS 20S PROTEASOME ACTIVITY
Fluorescent Intensity (U) at 440 nm
20
10
0
.0001
.001
.01
.1
1
10
100
DPBA (uM)

FIGURE 27

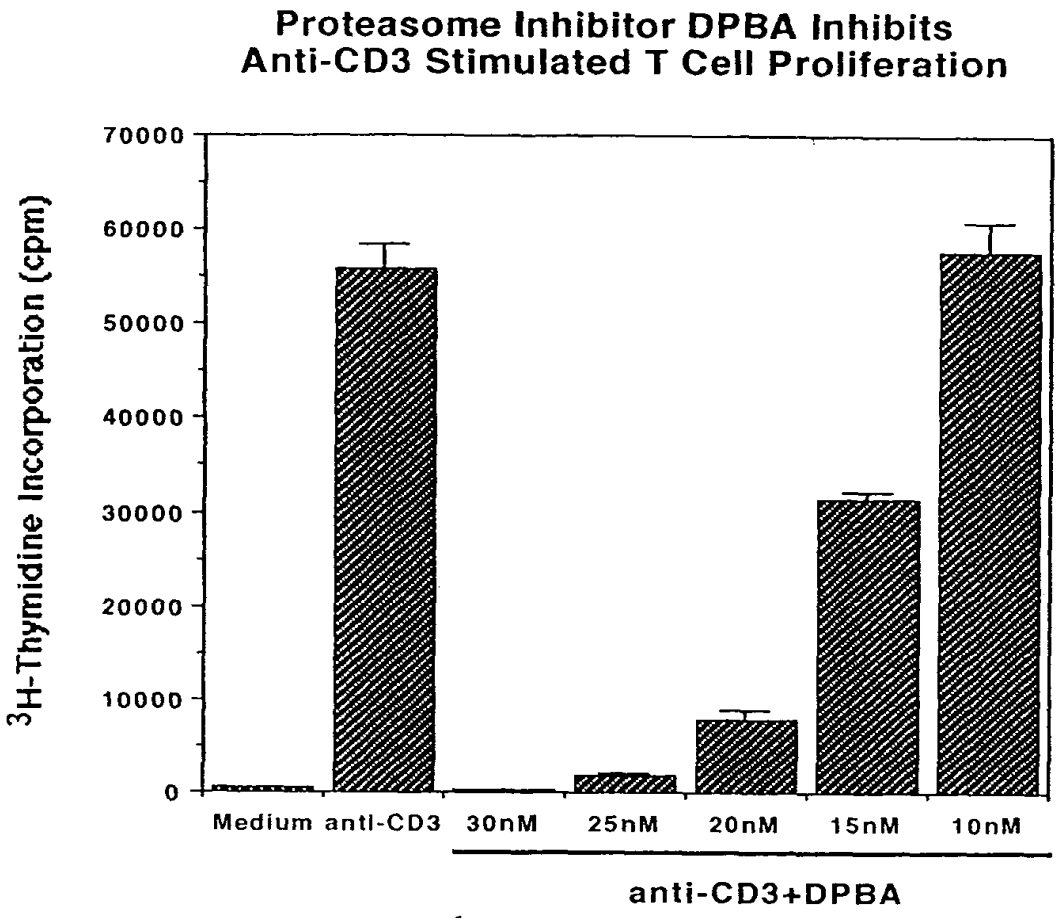
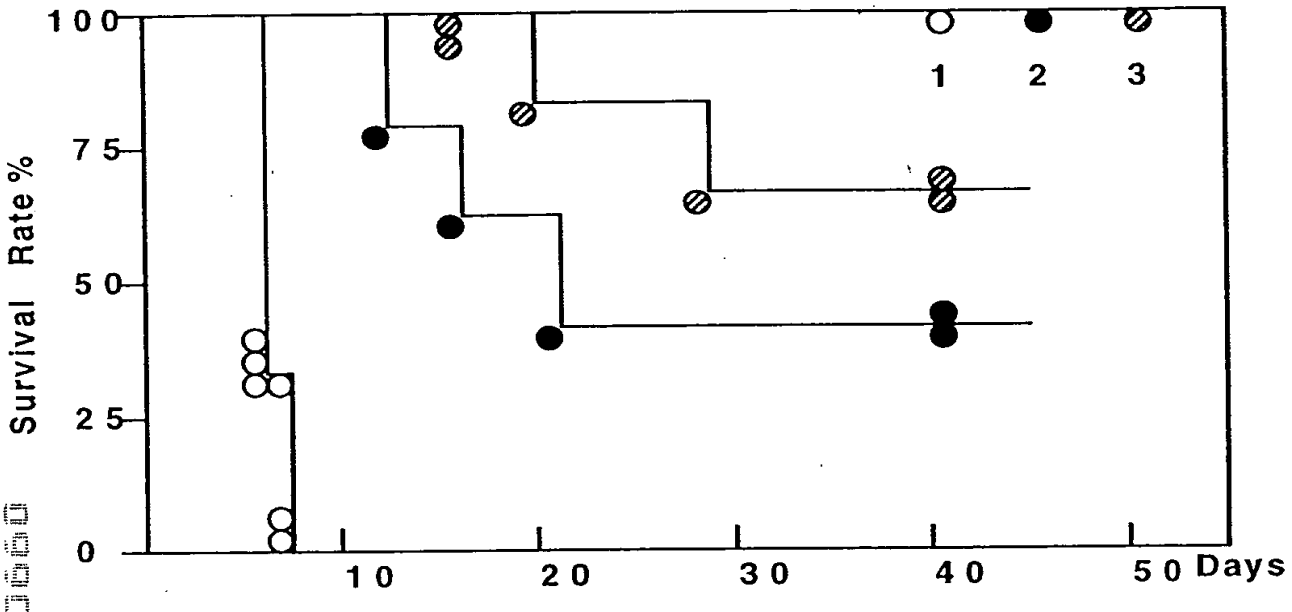

Proteasome Inhibitor DPBA Inhibits
Anti-CD3 Stimulated T Cell Proliferation
^{3}H-Thymidine Incorporation (cpm)
70000
60000
50000
40000
30000
20000
10000
0
Medium
anti-CD3
30nM
25nM
20nM
15nM
10nM
anti-CD3+DPBA

FIGURE 28

Proteasome Inhibitor DPBA Prolongs Mouse Heart Allograft Survival



	Groups	Survival Days	MST ± SD	p value
1	Control	7, 7, 7, 7, 8, 8	7,3 ± 0.5	–
2	DPBA 0.65mg/kg x 16 days	13, 16, 21 >40, >40, >16	>26.1 ± 13	0.006
3	DPBA 1.0mg/kg x 4 days, then 0.5mg/kg x 12 days	20, 29,>40 >16, >16 >16	>22.8 ± 9.8	0.008

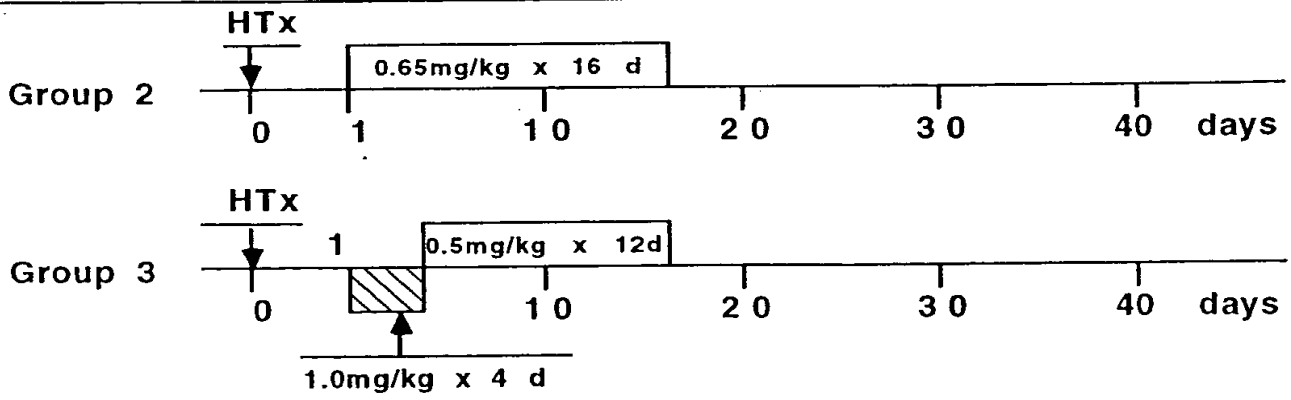
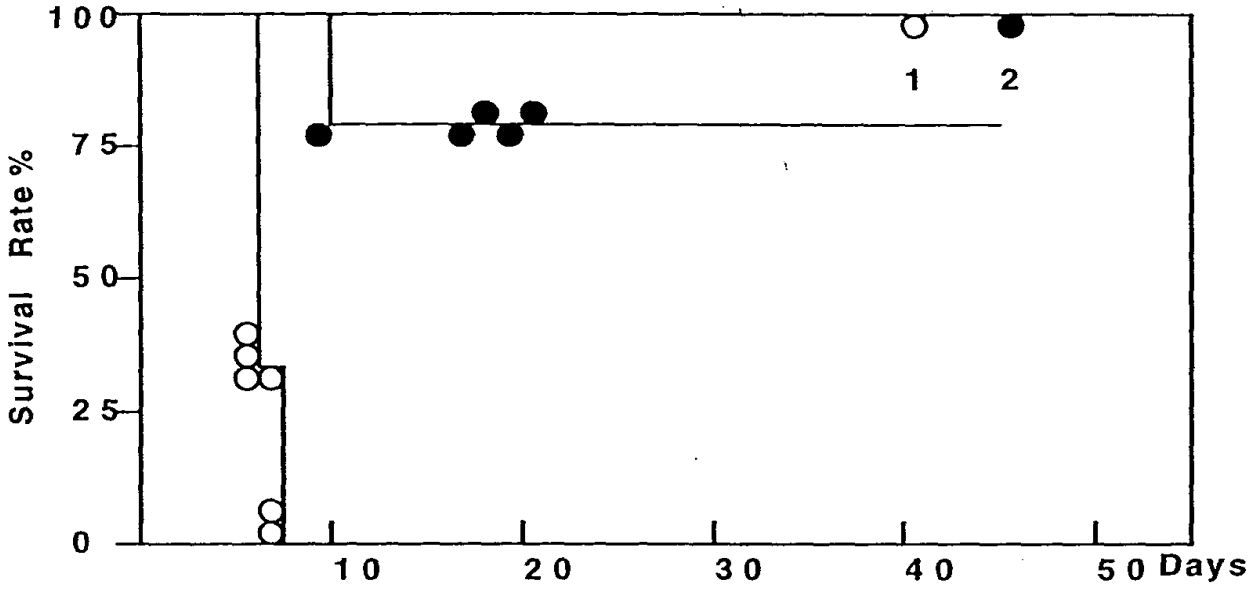


FIGURE 29

Proteasome Inhibitor DPBA is Effective in Treating Ongoing Heart Allograft Rejection in Mice



Groups	Survival Days	MST ± SD	p value
1 Control	7, 7, 7, 7, 8, 8	7,3 ± 0.5	–
2 DPBA 1.0mg/kg/day i.p. x 4 days	10,>14,>14 >14, >14,	>13.2 ± 1.78	0.0001

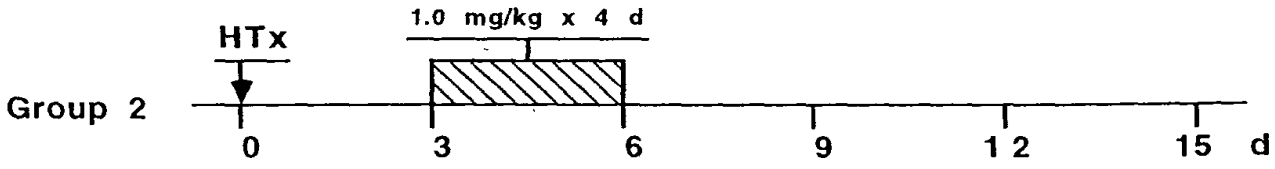


FIGURE 30

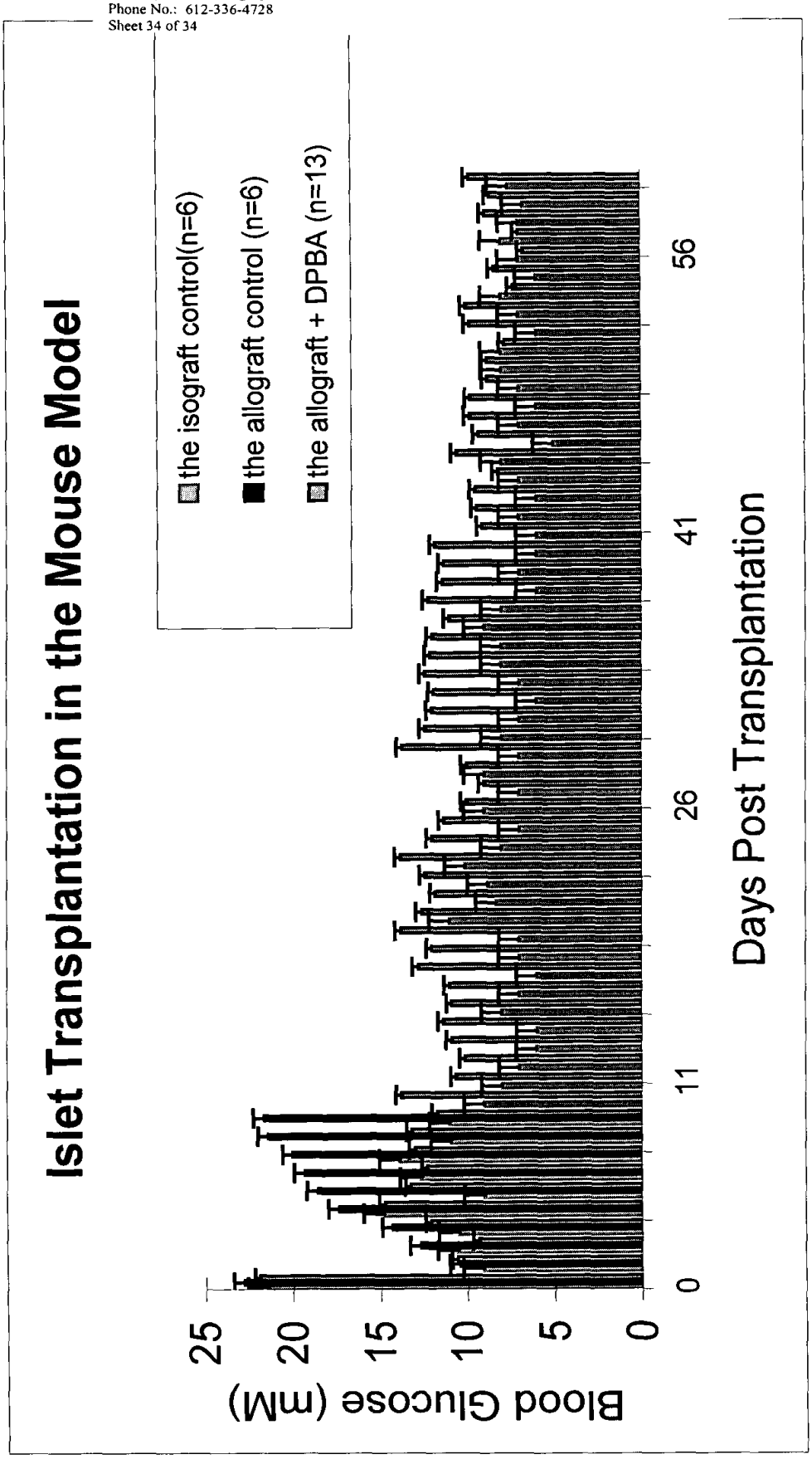

Islet Transplantation in the Mouse Model
the isograft control(n=6)
the allograft control (n=6)
the allograft + DPBA (n=13)
Blood Glucose (mM)
25
20
15
10
5
0
0
11
26
41
56
Days Post Transplantation

Figure 31